HEMORRHAGIC DISEASES

PHOTO-ELECTRIC STUDY OF BLOOD COAGULABILITY



HEMORRHAGIC DISEASES

PHOTO-ELECTRIC STUDY OF BLOOD COAGULABILITY

BY

KAARE K. NYGAARD, M.D.

Former Fellow in Surgery, the Mayo Foundation;
Former Assistant Surgeon, the University Clinic, Oslo;
Fellow of the Alexander Malthe Foundation
for Research in Medicine, Surgery and Gynecology

ILLUSTRATED

ST. LOUIS
THE C. V. MOSBY COMPANY
1941

Printed in the
United States of America

*Press of
The C. V. Mosby Company
St. Louis*

TO

THE MEMORY OF

WILLIAM JAMES MAYO

AND

CHARLES HORACE MAYO

IN GRATITUDE

FOR THE ACCEPTANCE AT THE MAYO FOUNDATION

OF FOREIGN STUDENTS

WHO UNDER THEIR GUIDANCE,

TOGETHER WITH THE NATIVE SONS OF THE UNITED STATES OF AMERICA,

ENJOYED THE PRIVILEGE

TO BENEFIT BY AND TO WITNESS

THE GROWTH AND FLOWERING OF A SEAT OF GREAT MEDICAL LEARNING

PREFACE

My first attempts through personal investigations to learn something about blood coagulation date back to 1931, shortly after my entrance as Fellow in Surgery at the Mayo Foundation. I have learned only slowly and have had to keep at the work continuously.

The investigations to be included in the present work were begun in 1935, with the application of the photo-electric principle to the measurements of the velocity of blood coagulation

The practical problems frequently confronting the surgeon and his assistants through their handling of patients with manifest or impending hemorrhage have throughout induced a constant stimulation to further investigations

Originally attacking the problem from this practical and clinical point of view, I soon realized that technical and clinical problems of hematologic nature necessarily had to be viewed in intimate relation to the physiology of blood coagulation With the constellation, however, of these three sets of problems with each of them harboring numerous imponderables, one is forced to juggle with three balls, two of which are constantly up in the air. This, no doubt, has added to the complexity and no less to a certain degree of confusion characterizing the problem of blood coagulation.

The idea of breaking the interlocking of these three sets of related problems by concentrating on one of them has been obvious to anyone confronted with the mystery of blood coagulation. To most of us the logical approach has been a concentration on the measurement of the velocity of blood coagulation. For years this has constituted a routine laboratory procedure Yet, these measurements are complicated for one main reason. The velocity to be measured is only a relative factor and variable in relation to the measuring principle. The success and the consequence of these measurements will depend primarily upon the realization of this relationship, upon means of analyzing it, and upon the stabilization of most or all of the variable factors

With the introduction of the photo-electric principle to the present problems I believe we have at our disposal a technique offering a more dependable and more complete analysis of the various phases of the process of blood coagulation and its velocity

It has been my object in the first part of the present work to present an outline of various approaches to the measurement of blood coagulation, to describe the photo-electric principle and its applicability to problems related to medical investigations in general and to the problem of blood coagulation in particular, and further to present a detailed analysis of factors influencing the velocity of blood coagulation crystallizing in standardized methods for investigation of the coagulability of the blood

It has been impossible to perform a study of the present type without due consideration of problems of primary physiologic interest This has been done

in the realization that there are no short cuts to be made if the complexity of the present problem is ever to be simplified.

The excellent and most fruitful researches of recent years by Dam, by Quick, by Smith, Brinkhous, and Warner, by Butt and Snell, and by many others concerning the therapeutic applicability of vitamin K in cases with deficiency of prothrombin have created an ever-widening interest in the quantitative estimation of prothrombin. An essential part of the present work leads to a detailed analysis of the nature and validity of these methods for quantitative prothrombin estimation.

With standardized investigative methods I have proceeded to a fairly large clinical group comprising the main types of hemorrhagic diseases. This has afforded the opportunity of demonstrating the importance of the second stage of the investigative technique. Known hemorrhagic disorders are used as the yardstick for the merits of the various investigative methods These in turn are employed as essential additional aids in the diagnosis, prognosis, and therapy of the clinical cases. It is admittedly a long road from the urgent bedside problems to the conclusions of what may and may not be accomplished at the present time with various therapeutic procedures The researches of recent years centering on vitamin K have opened entirely new fields Essential problems still remain. I hope in the discussion of the clinical problems to have ended up with the right kind of deficient knowledge, namely, that which is occasionally constructive.

I am naturally not in the position of proceeding to the third stage of the investigative technique dealing with central problems of the physiology of blood coagulation. This must be left to the expert physiologist. I have harbored the hope that the investigative approach presented in this work may indicate the applicability of the photo-electric technique to central physiologic problems of blood coagulation, like the rate of thrombin production and the nature of thrombin.

It must be a great satisfaction to everyone interested in the progress of medicine to sense the great forward stride in this particular field during the last few years. In fact, a series of valuable contributions has been presented in such rapid sequence as to make it increasingly difficult to offer a more complete exposition of our investigations and simultaneously to include an up-to-date review of parallel investigations by other workers The review of the literature as presented in this work covers only the main events to June, 1940. For this shortcoming I ask the indulgence of the authors of a great number of subsequent contributions.

During the execution of these investigations I have to a great extent drawn on the good will, patience, and experience of a great number of colleagues and friends whom I sincerely hope may have sensed my appreciation for their services. Among the staff members of the Mayo Clinic I beg to mention:

Dr. Waltman Walters, in whose surgical department I had the good fortune to serve as assistant and in cooperation with whom I received constant encouragement, enlightenment, and access to abundant clinical material.

Dr. D. C. Balfour and Dr. L. B. Wilson, directors of the Mayo Foundation, who have repeatedly and patiently listened to a great number of plans, many of them not brilliant, and who invariably left with me valuable corrections and wise suggestions.

Dr. C. A. Sheard and Dr. E. J. Baldes, of the Department of Physics and Biophysical Research, with whose cooperation the first apparatus was made for the photo-electric investigations of blood coagulation.

Dr. A. H. Sanford, in whose laboratory I obtained all the facilities for the performance of an important part of these investigations.

Dr. J. deJ. Pemberton, Dr. H. Z. Giffin, and Dr. A. M. Snell and the members of their departments, from whom I received a great number of cases for observation.

Miss Dora Schellin, chief technician of the laboratory, faithful and resourceful co-worker.

Among the members of the staff of the University Clinic, Oslo, I mention with devotion my former chiefs during the last three years: Professor Johan M. Holst and Professor Anton Sunde, whose constant encouragement, stimulation, and generosity have added greatly to the completion of this work. I should also like to acknowledge the valuable cooperation freely given by Dr. A. Falck during the construction of the portable photelgraph and the constructive criticism and cooperation of Dr. Th. Guthe.

To all these colleagues I beg to express my sincere gratitude for their cooperation and participation in these investigations.

It is my pleasure to acknowledge the kind assistance extended to me by the pharmaceutical houses of Hoffmann-La Roche, Basel, Switzerland, and the Glaxo Laboratories, Ltd , Greenford, England, who have been very generous in supplying me with their latest vitamin K products, and also of Nyegaard & Company, Oslo, for their supply of the thromboplastic material.

To the trustees of Alexander Malthe's Legacy, Oslo, my humble thanks for their unqualified backing and most generous grants.

KAARE K. NYGAARD.

Rochester, Minn.

CONTENTS

PART I

CHAPTER I

PAGE

CHAPTER II

CHAPTER III

CHAPTER IV

CHAPTER V

PART II

CHAPTER VI

CHAPTER VII

CHAPTER VIII

PART III

CHAPTER IX

CHAPTER X

CHAPTER XI

CHAPTER XII

CHAPTER XIII

CHAPTER XIV

ILLUSTRATIONS

HEMORRHAGIC DISEASES

PHOTO-ELECTRIC STUDY OF BLOOD COAGULABILITY

HEMORRHAGIC DISEASES

PHOTO-ELECTRIC STUDY OF BLOOD COAGULABILITY

PART I

CHAPTER I

METHODS OF DETERMINING THE COAGULABILITY OF THE BLOOD

Introduction

It has been stated by a worker well versed in natural sciences that "when you can measure what you are speaking about and express it in numbers, you know something about it" (Lord Kelvin). One may with equal justification add that in order to perform a measurement one must know something about that which is to be measured.

The above statement was made after I had studied numerous methods for the determination of the so-called coagulation time of the blood. These methods have resulted in innumerable measurements during the last half century, and expressed in numbers at that. Truly, we have learned something about the matter. Compared with the enormous advances of other problems of biologic nature during the same interval, one is tempted to state that the advance made does not stand in any reasonable relation to the enormous amount of work performed. Have we measured without knowing enough about the matter to be measured?

There may be several relevant excuses for this state of affairs. The blood as a most important part of the fluid constituents of the living organism is a very labile material for in vitro investigations. In vivo this is quite a different matter The maintenance of the liquid state of the blood in vivo is a condition sine qua non for the continuance of life. The closer mechanism governing and regulating the fluidity of the blood is no doubt an intricate problem, in the knowledge of which important links are still missing. It may suffice in this connection to mention the present inadequate knowledge regarding the activity of the liver, spleen, and bone marrow as organs of significance for this mechanism The physiologic process of blood coagulation is a complex problem, a fuller understanding of which is still lacking. It may be reasonable to suppose that the problem of the fluidity of the blood may prove to be non-identical to the question of the physiologic process of coagulation of the blood.

Let us for a moment assume that these problems were solved; furthermore, that one or several factors known to be essential for the fluidity of the blood were found, for instance, to be of chemical nature. Assuming that quantita-

quence that this fact necessarily excludes the conception of a "normal" coagulation time per se. Such a term is as contradictory as the above-mentioned "physiologic" coagulation time. A coagulation time of 2 minutes with one technique may be just as normal as one of 30 minutes with another. In other words, the methods with which we are concerned here are of quite a different order than quantitative biologic methods as represented, for instance, by procedures for determination of the oxygen saturation of erythrocytes or for investigations of the acid-base equilibrium of the blood. A comparison of various methods for determination of the coagulability of the blood consequently cannot be undertaken on the basis of the coagulation time per se. When such an evaluation is desirable, we are left with a comparison of the various methods on the basis of their merits in clinical and experimental fields

Let us now return to the sentences introducing the present chapter. It is my problem to justify my reservation to the honored scientist's truly stimulating statement. This may be done in the form of a definition which may indicate about what we are speaking

The coagulation time of the blood is a relative expression of the potential ability of the blood to be transformed in vitro from the liquid to the clotted state under the influence of an extrinsic, nonhematologic factor or factors

Survey of Methods

With the preceding remarks the intention has been to obtain a background for a survey of the various methods employed in determining the coagulability of the blood. I have been anxious to convey an impression of what may, and particularly of what may *not*, be expected from these estimations, which from a scientific point of view surely must be considered inferior to many other methods employed in the medical laboratory It has been repeated to the point of monotony that the very number of methods published is an expression of this unsatisfactory state of affairs I, at least, feel that the excessive number of methods should not be sufficient to paralyze further interest in this problem. Each method may have added something to the clarification of this complex problem The stepping stones of progress are unknown Stimulation in the present instance is furnished constantly by the serious clinical conditions with which these investigations are closely connected. When it is further remembered that investigations of the physiologic problem of the coagulation of the blood are partly dependent on methods for studies of the coagulant or anticoagulant principles under varying experimental conditions, an impression is gained of the pressing problems attacked by the procedures at present available, however inefficient they may have been

In attempting to present a survey of available methods for determination of the coagulability of the blood, it will be readily understood that little will be gained by presenting an outline of each method with a detailed technique. Such an outline falls within the scope of textbooks on hematologic technique. When considering this survey from an opposite view, that is, reviewing the various methods not according to their differences but according to their com-

tive methods were available for the determination of these factors, the fluidity of the blood may hypothetically be expressed in the gravimetric system Such an assumption may appear unlikely. To the older clinicians, however, it may likewise have sounded like a foggy philosophy of expressing the sweetness of *diabetic urine in milligrams of something so long as the only thing they knew* about that urine was that it was sweet to the taste. It was no long way from the registration of the sweet taste of the urine to a clear conception of an acting agent producing this sensation, *and further on to chemical methods for* the quantitative determination of sugar in urine and in blood.

The maintenance of the fluidity of the blood has proved to be a problem of different magnitude. No principle is available for a direct estimation of this fluidity. The numerous methods for determination of the coagulability of the blood are concerned with the reverse side of this problem; namely, an investigation of the clot-forming ability of the blood. This type of investigation *is suggested by nature itself. Clinicians and laymen throughout the ages have observed that the blood of some individuals, after escaping the human* body, remains in a liquid state longer than that of other individuals. The person was considered "prone to bleed." In such a case a laboratory examination, *in* its embryonic form, was coupled with clinical observation. With some modifications, the same investigative principle is maintained today. From the measurement of the clot-forming ability of the blood we are reasoning back *to the state of the blood in vivo This principle probably ought to be accepted* only with reservations Our measurements mean no specific determination of factors governing clot formation *In units of time the measurements express the resultant activity of coagulant principles, known and unknown, of intrinsic hematologic as well as of purely external extrahematologic nature.* These measurements can have only indirect relation to the properties of the blood in vivo Blood withdrawn without the coagulant effect of external influences would be expected, theoretically, to keep in a liquid state identical to that of blood in vivo. When considering coagulation of the blood, it is therefore necessary to allow for extrahematologic, external factors of one type or another For this reason, it appears irrelevant to talk about the "physiologic" coagulation time of the blood, an expression occasionally encountered in medical literature *Coagulation is not a vital property of the blood but is a potential property It is this potential property which we estimate with our various methods.*

If, in a coagulating specimen, the intrinsic hematologic factor is considered as A, and the external factor as B, the resultant coagulation measured in time C is a result of the interaction of A and B It is obvious that if a method were able to dispense with all external factors, that is $B = 0$, it would cease to be a method. *External coagulant* influences also have to be taken into account. The problem is to keep this factor constant (B = constant K), thereby permitting a relative estimation of A If it were possible to work with a constant dosage of B, the ideal method would be found The hope of any present method can be only to approach this ideal state From a theoretic point of view the value of B may be arbitrary, if only constant It is a natural conse-

The following is a classification of methods for determination of the coagulability of the blood:

1. Mechanical removal of fibrin threads (Vierordt, Sabrazès).
2. Observation of change of contour of hanging drop (Milian).
3. Observations of arrest of movements of intrinsic or extrinsic particles in the blood (Brodie and Russell, Fuld).
4. Observations of surface of blood when exposed to various continuous or interrupted mechanical influences in capillary tubes and larger vessels (Morawitz and Bierich, Belák).
5. Direct viscosimetry (Kottmann).
6. Optical technique: (a) direct microscopy (Schwab, Kitamura), (b) transillumination (Kugelmass).

A classification of this type may be convenient for a critical review of reading technique. The feeling, however, prevails that future progress of this problem will not depend primarily on a continuation of the discussion of end points in its present form

I have tried to consider the problem from a different angle. When blood is clotting, this process progresses parallel with a progressive change in its relative viscosity. The relative viscosity of freshly drawn blood is dependent on the concentration of the total proteins, the viscosity of the dispersion fluid, the interrelation of the concentration of the different proteins, the colloidal state of the various proteins, the concentration of the corpuscular elements, and the temperature.[2, 3]

At the time of fibrin formation, fundamental changes have occurred in the colloidal state of the fibrinogen. This transition is accompanied by a marked increase in the relative viscosity of the specimen. Regardless of whether most of the various methods considered above are consciously concerned with a reading of this relative increase of viscosity, it appears nevertheless that most of the methods in one way or another are directly or indirectly dealing with this increased viscosity of the blood, further, that this change produces their end point. There is no fundamental difference in the principle, whether the change of viscosity is demonstrated by a foreign body introduced into the blood (horsehair), by particles suspended in or introduced into the blood (erythrocytes, glass beads), or by the material surrounding the blood (glass wall) It is further clear that the change in contour of a drop of blood on a glass slide during the repeated tilting of the slide from a horizontal to a vertical position can be viewed from the same angle, the result of an increasing viscosity between the layers in the interior of the drop

From the physical point of view a classification of the various methods* for the coagulability of the blood may be arranged as follows:

A Methods based on viscosimetry
  1. Indirect viscosimetry
  2 Direct viscosimetry.

*One method, that of van Allen, cannot be classified according to the above indicated principle. The end point of this method is indicated by the beginning of expression of serum from an individual drop of whole blood. It is clear that this end point is fundamentally different from the two end points with which most of the other methods are concerned.

mon characteristics or principles, one may arrive at some constructive knowledge regarding the subject.

When blood in vitro is transformed from the liquid to the clotted state, the transition is the result of formation of the protein fibrin. This clot formation requires a certain length of time. In attempting to measure this clotting time, the zero point for these measurements is readily agreed upon. Great difficulty has been encountered in deciding upon the end point of the reaction. In fact, it appears that the struggle for accurate methods has centered on the problem, in one way or another, of producing the sharpest possible end points Fonio[1] has defined two such important end points, both to be considered by more efficient methods. These two points are represented by the moment the fibrin first appears and the moment the clot is completely formed. To these measurements Fonio applied the terms "*Reaktionszeit*" and "*Gerinnungszeit*," respectively. From theoretic and practical points of view these end points of Fonio must be considered definite. The formation of fibrin is in itself a time-consuming process and not on the order of a sudden precipitation. The onset of fibrin formation precedes the transition of the specimen into a firm clot.

From a practical point of view the difficulty is to detect one or both of these end points. From what is stated above, the coagulation of the blood may be regarded as a reaction in progression until formation of the clot. (Investigations to be dealt with later indicate that this progression continues even past the formation of a clot.) Attempts to follow this progression of the process can be made in two ways, either by intermittent, frequent observations during the process or by more or less continuous observation during the process. An example of the first form of arrangement is represented by a horizontally placed capillary tube containing the blood, which at intervals is tilted toward the vertical position, whereby blood intermittently will escape the lower end of the tube until clotting is completed. The second type is represented by a vertically placed capillary tube that permits the continuous dripping of blood from its lower end until interrupted by clotting of the blood. Under otherwise equal conditions it is apparent that methods permitting continuous observation of the reaction are of advantage In the literature it is generally understood that a method permitting the reading of both the beginning of fibrin formation and the point of completed clot formation is superior to one in which the reading of only one of these points can be made. For research purposes this conception may hold true. For clinical purposes the question seems more doubtful. As will be taken up in a later chapter, there exists a certain constant relation between the "*Reaktionszeit*" and "*Gerinnungszeit*"; further, the determination of both appears to give no additional clinical information to that obtained by the determination of only one of these values.

After this short exposition of reading technique, it appears natural to survey the available methods from the same point of view. The intimation given above, that the development of technique for the determination of coagulability of the blood for a greater part has been concerned with improvements in reading technique, is then clearly brought out.

The following is a classification of methods for determination of the coagulability of the blood:

1. Mechanical removal of fibrin threads (Vierordt, Sabrazès).
2. Observation of change of contour of hanging drop (Milian).
3. Observations of arrest of movements of intrinsic or extrinsic particles in the blood (Brodie and Russell, Fuld).
4. Observations of surface of blood when exposed to various continuous or interrupted mechanical influences in capillary tubes and larger vessels (Morawitz and Bierich, Belák).
5. Direct viscosimetry (Kottmann).
6. Optical technique: (a) direct microscopy (Schwab, Kitamura), (b) transillumination (Kugelmass).

A classification of this type may be convenient for a critical review of reading technique. The feeling, however, prevails that future progress of this problem will not depend primarily on a continuation of the discussion of end points in its present form.

I have tried to consider the problem from a different angle. When blood is clotting, this process progresses parallel with a progressive change in its relative viscosity. The relative viscosity of freshly drawn blood is dependent on the concentration of the total proteins, the viscosity of the dispersion fluid, the interrelation of the concentration of the different proteins, the colloidal state of the various proteins, the concentration of the corpuscular elements, and the temperature.[2, 3]

At the time of fibrin formation, fundamental changes have occurred in the colloidal state of the fibrinogen. This transition is accompanied by a marked increase in the relative viscosity of the specimen. Regardless of whether most of the various methods considered above are consciously concerned with a reading of this relative increase of viscosity, it appears nevertheless that most of the methods in one way or another are directly or indirectly dealing with this increased viscosity of the blood, further, that this change produces their end point There is no fundamental difference in the principle, whether the change of viscosity is demonstrated by a foreign body introduced into the blood (horsehair), by particles suspended in or introduced into the blood (erythrocytes, glass beads), or by the material surrounding the blood (glass wall) It is further clear that the change in contour of a drop of blood on a glass slide during the repeated tilting of the slide from a horizontal to a vertical position can be viewed from the same angle, the result of an increasing viscosity between the layers in the interior of the drop.

From the physical point of view a classification of the various methods* for the coagulability of the blood may be arranged as follows.

A Methods based on viscosimetry.

1. Indirect viscosimetry
2. Direct viscosimetry.

*One method, that of van Allen, cannot be classified according to the above indicated principle The end point of this method is indicated by the beginning of expression of serum from an individual drop of whole blood. It is clear that this end point is fundamentally different from the two end points with which most of the other methods are concerned.

mon characteristics or principles, one may arrive at some constructive knowledge regarding the subject.

When blood in vitro is transformed from the liquid to the clotted state, the transition is the result of formation of the protein fibrin. This clot formation requires a certain length of time. In attempting to measure this clotting time, the zero point for these measurements is readily agreed upon. Great difficulty has been encountered in deciding upon the end point of the reaction. In fact, it appears that the struggle for accurate methods has centered on the problem, in one way or another, of producing the sharpest possible end points. Fonio[1] has defined two such important end points, both to be considered by more efficient methods. These two points are represented by the moment the fibrin first appears and the moment the clot is completely formed. To these measurements Fonio applied the terms "*Reaktionszeit*" and "*Gerinnungszeit*," respectively. From theoretic and practical points of view these end points of Fonio must be considered definite. The formation of fibrin is in itself a time-consuming process and not on the order of a sudden precipitation. The onset of fibrin formation precedes the transition of the specimen into a firm clot.

From a practical point of view the difficulty is to detect one or both of these end points From what is stated above, the coagulation of the blood may be regarded as a reaction in progression until formation of the clot. (Investigations to be dealt with later indicate that this progression continues even past the formation of a clot.) Attempts to follow this progression of the process can be made in two ways, either by intermittent, frequent observations during the process or by more or less continuous observation during the process. An example of the first form of arrangement is represented by a horizontally placed capillary tube containing the blood, which at intervals is tilted toward the vertical position, whereby blood intermittently will escape the lower end of the tube until clotting is completed The second type is represented by a vertically placed capillary tube that permits the continuous dripping of blood from its lower end until interrupted by clotting of the blood. Under otherwise equal conditions it is apparent that methods permitting continuous observation of the reaction are of advantage. In the literature it is generally understood that a method permitting the reading of both the beginning of fibrin formation and the point of completed clot formation is superior to one in which the reading of only one of these points can be made. For research purposes this conception may hold true. For clinical purposes the question seems more doubtful. As will be taken up in a later chapter, there exists a certain constant relation between the "*Reaktionszeit*" and "*Gerinnungszeit*"; further, the determination of both appears to give no additional clinical information to that obtained by the determination of only one of these values.

After this short exposition of reading technique, it appears natural to survey the available methods from the same point of view. The intimation given above, that the development of technique for the determination of coagulability of the blood for a greater part has been concerned with improvements in reading technique, is then clearly brought out.

6 Brodie and Russell[18] (1897)	Venous or capillary	Hanging drop on glass slide	Satisfied	–	+	+	3 8	Specially designed instrument, *coagulometer;* hanging drop examined under microscope, erythrocytes set in motion by stream of air intermittently blown on edge of drop; end point when movements of erythrocytes are arrested	Boggs[16] (1908) Addis[2] (1909) Fuchs[17] (1930)
7 Buckmaster[19] (1907)	Venous or capillary	Wire loop	Satisfied (30 40° C)	–	+	–	8	Blood film in wire loop; intermittently loop changed from horizontal to vertical position; movements of erythrocytes observed with magnifying glass; end point when arrest of movements	Inchley[19] (1921) Gibbs[20, 21] (1924)
8 Biffi[22] (1904)	Venous or capillary	Wire loop	Satisfied (20 25° C)	–	+	–	7 10	Blood film in five wire loops which at intervals consecutively are introduced into water; end point when blood not diffusing into the water	
9 Fuld and Schlesinger[13] (1912)	Venous	U formed tube with metal bead	Satisfied	–	+	–	4	Specially designed apparatus, *thrombometer;* by tilting U formed tube, a metal bead moves back and forth until arrested by clot formation	Hedenius[24] (1936)
10 Szankowski[25] (1937)	Venous	Glass tube	Satisfied	–	+	+	--	By special arrangement small air bubble blown through blood *once per second;* end point when air bubble not ascending	

TABLE I

METHODS FOR DETERMINATION OF THE COAGULABILITY OF THE BLOOD

GROUP	AUTHOR	TYPE OF BLOOD	TYPE OF CONTAINER	TEMPERATURE REQUIREMENTS	END POINT		CONTINUOUS OBSERVATION	AVERAGE COAGULATION TIME (MIN.)	TECHNIQUE	MODIFICATIONS BY
					FIRST APPEARANCE OF FIBRIN	CLOT FORMATION				
AI	1 Vierordt[6] (1878)	Capillary	Capillary glass tube	Not considered	+	+	–	9 10	White horsehair drawn through blood; first end point when shreds of fibrin on hair, second point when hair again clean	Kottmann and Lidsky[5] (1910)
	2 Burker[6, 7] (1904)	Capillary	Glass slide	Satisfied (18° C.)	+	–	–	6 7	Blood at intervals stirred gently with fine glass rod until appearance of first fibrin threads	Perrin and Hanns[8] (1922)
	3 Kaufmann[9] (1925)	Venous	Specially designed small test tubes	Satisfied	+	–	–	—	Instrument termed *blood-coagulo-tachymeter*; platinum wire at *intervals* dipped into and lifted out of blood until appearance of fibrin thread	
	4 Sabrazès[10] (1904)	Capillary	Capillary glass tube	Considered	+	–	–	9-10	Use *three* capillary tubes, pieces of which at intervals are broken off until appearance of first fibrin thread	McGowan[11] (1907) Addis[12] (1909) Petersen and Mills[13] (1923)
	5 Schultz[14] (1910)	Capillary	*Hohlperlen-kapillare*	Satisfied	+	+	–	9 10	Special capillary tube consisting *of 3* to 12 hollow beads which at intervals are broken off and placed in NaCl solution; when fibrin formed, shreds *of it* will float in solution; whereas, solution remains clear when blood clotted	

16. Práwdicz Neminski[38] (1927)	Capillary	20 to 25 capillary tubes	Satisfied	+	+	–	—	25 capillaries, 8 mm. long, 1 mm. wide, filled with blood and placed in individual containers filled with saline solution and in vertical position; end point when no more blood escapes	
17 Heubner and Rona[39] (1923)	Venous	Special pipette	*Satisfied* (20-30° C.)	–	+	+	4-4½	Apparatus termed *coagulometer;* vertically placed capillary pipette from *which* blood dripping by gravity; end point when dripping ceases	
18 Frisch and Starlinger[40] (1921)	Venous	Capillary tubes	Satisfied (20° C.)	+	+	–	15 20	Apparatus with capillaries raised intermittently from horizontal to vertical position; end point when fibrin adheres to the glass, further when flow of blood ceases	Homma[41] (1930) Trought and Riddoch[42] (1936) van Walsem[43] (1928)
19 Lampert[44, 45] (1930)	Venous	Special pipette	Satisfied (37° C.)	+	+	+	30	Pipette placed obliquely; similar technique to 17; important modification is that material is made from athrombit	
20. Belák[46] (1919)	Venous	Special pipette	*Satisfied* (30° C.)	+	+	–	4 5	Blood column intermittently pressed up into vertically placed viscosimeter tube; end point when column not moved by pressure of 1 cm. water	Geers[47] (1921) Jegorow[48] (1922) Feissly[49] (1922) Aggazotti[50] (1933)

TABLE I—CONT'D

GROUP	AUTHOR	TYPE OF BLOOD	TYPE OF CONTAINER	TEMPERATURE REQUIREMENTS	END POINT		CONTINUOUS OBSERVATION	AVERAGE COAGULATION TIME (MIN.)	TECHNIQUE	MODIFICATIONS BY
					FIRST APPEARANCE OF FIBRIN	CLOT FORMATION				
	11. Hayem[26] (1898)	Venous	Flat-bottomed test tube	Not considered	–	+	–	3-20	Flat bottomed test tube filled with 3 c.c. whole blood; tilted at intervals; end point when tube tilted without changing the level of surface	Lee and White[27] (1913)
	12. Milian[28] (1901)	Capillary	Glass slide	Not considered	–	+	–	10-15	At intervals glass slide with drop of blood tilted from horizontal to vertical position; end point when contour of drop remains convex	Hinman and Sladen[29] (1907) Duke[30] (1910) Cohen[31] (1911) Pétren[32] (1920)
	13. Morawitz and Bierich[33] (1907)	Venous		Satisfied (20° C.)	+	+	–	15 20	5 c.c. blood deposited in special container which is gently tilted at intervals; end point at appearance of red layer on glass wall, as well as maintained surface level when tilted	Wohlisch[34] (1922) Fonio[1] (1928)
	14. Wright[35] (1893)	Capillary	Capillary tubes	Satisfied (18.5° C.)	–	+	–	2-4	3 to 4 capillaries filled with blood; at intervals blows at one end of capillary; end point when no more blood coloring filter paper	Yatsushiro[36] (1913)
	15. Mas y Magro[37] (1924)	Capillary	Paraffined glass slide	Satisfied (15° C.)	+	+	–	8-12	Drop of blood on paraffined glass slide; at intervals blood sucked into paraffined capillary; end point when no blood flows into capillary	

	4 Takenaka[58] (1932)	Venous	Glass cup	Satisfied	+	+	+	--	Specially designed apparatus, *coagulo viscosimeter;* coagulation curve plotted on the basis of readings of the viscosimeter; changes can also be photographically recorded	
BI.	1 Schwab[60] (1906)	Capillary	Glass slide	Not considered	+	-	+	5 0	Hanging drop of blood is microscopically examined under oil immersion lens; end point is first appearance of fibrin	
	2 Kitamura[61] (1924)	Citrated plasma	Glass slide	Considered	+	+	+	--	Citrated plasma brought to coagulation by recalcification or dilution; specimen observed microscopically through dark field technique; end point first formation of fibrin	
	3. Kugelmass[59] *(1923)*	Oxalated *plasma*	Glass cuvette	Satisfied	+	+	+	--	Specially designed apparatus, *nephelometer; based on* variation of transparency during coagulation	
BII	4 Klinke and Elias[62-65] (1931)	Oxalated plasma	Specially designed glass tubes	Satisfied	+	+	+	--	During formation of fibrin changes occur in the dispersion of the system resulting in a simultaneous variation in the Tyndall effect, which is studied by a Stufen photometer	
	5 Baldes and Nygaard[66] (1936)	Oxalated plasma	Special absorption cells	Satisfied (37.5° C.)	+	+	+	3 4	Specially designed apparatus, (1) *coagelometer*, and (2) *coagelgraph;* based on photoelectric observations of variations of transmitted *light during coagulation*	

TABLE I—CONT'D

GROUP	AUTHOR	TYPE OF BLOOD	TYPE OF CONTAINER	TEMPERATURE REQUIREMENTS	END POINT		CONTINUOUS OBSERVATION	AVERAGE COAGULATION TIME (MIN.)	TECHNIQUE	MODIFICATIONS BY
					FIRST APPEARANCE OF FIBRIN	CLOT FORMATION				
	21. Takasaki[51] (1923)	Capillary	Fine capillary tube	Satisfied	+	+	–	3-5½	Horizontally placed capillary in which intermittently the blood column is moved by turning a screw; end points indicated by uneven meniscus, further by arrest of blood column	Szabunewicz[52, 53] (1932) Riesser and Nagel[54] (1935) Szankowski[25] (1937)
AII	1 Cannon and Mendenhall[55] (1914)	Capillary	Special capillary glass tube	Satisfied	+	+	–	6-10	Apparatus termed *coagulometer;* copper wire fastened to short arm of suspended lever, and at intervals inserted into blood in a special tube; long arm of lever records movement on smoked drum; after appearance of fibrin, movements of long arm increasingly inhibited	
	2. Kottmann[56] (1910)	Venous	Small metal container	Satisfied	+	+	+	15 20	Specially designed apparatus, *coaguloviscosimeter*, is a direct measurement of viscosity; coagulation-curve plotted on the basis of readings of the viscosimeter	
	3. Foster[57] (1931)	Venous	Blood between glass and aluminum plate	Satisfied	+	–	–	Dogs, 9 Rabbits, 11	Specially designed apparatus, *visco coagulometer;* records intermittently viscosity changes through kymographic arrangement	

B. Optical methods.
  1. Direct microscopy.
  2. Transillumination technique.
C. Van Allen's method.
D. Plasma coagulability tests.

On the basis of this classification a survey is given of the various methods in Table I with a brief indication of technique and points of principal interest. The survey does not pretend to represent a complete review of all published methods. Procedures are included only so far as a new principle or essential points of technical interest have been presented. Where a modification of a method has been worked out, this modification is included only in case of essential additions to the previously published method, and so indicated in the last column of the table. All other methods have been omitted. An historical review, however interesting and instructive it might be, has been left out. The year of publication of each method is included and chronologic order is maintained in the subgroups. A subdivision into smaller units has been undertaken to facilitate the survey not only according to identity in principle, but also according to similarity of technique. By this arrangement it may also be possible to convey the impression how surprisingly few have been the leads offered the research worker interested in this problem.

Comment

It was previously stated that fundamental links are missing for a deeper understanding of the physiologic process of the coagulation of the blood. This is particularly felt when trying to present a constructive critical review of the methods listed in the table. We are confronted here with the peculiarity of dealing with two partly separate problems, each of which is not completely understood, and for the explanation of which information regarding the one is derived from studies of the other.

In the subsequent exposition I have chosen to present my review in the form of a brief presentation of the more generally accepted observations bearing on the coagulation process, and further on the role of the platelets, particularly their importance for the inception of coagulation

As previously pointed out, coagulation of the blood in vitro is a result of the *interaction of intrinsic hematologic and extrinsic factors.* Without going into details regarding the physiology of this process, it may be stated that one of the more commonly accepted theories regarding this process is expressed briefly as follows:

1. Thrombokinase + calcium + prothrombin → thrombin.
2. Thrombin + fibrinogen → fibrin

Great interest has centered on the phenomena relative to inception of coagulation of the blood. A mass of experimental data has been presented with the object of elucidating the question whether the primary changes are to be found in the plasma or in the platelets. It is outside the scope of the present chapter to present a survey of these investigations. In addition, the

Table I—Cont'd

Group	Author	Type of Blood	Type of Container	Temperature Requirements	End Point: First Appearance of Fibrin	End Point: Clot Formation	Continuous Observation	Average Coagulation Time (Min.)	Technique	Modifications by
C.	1. Van Allen[67] (1927)	Capillary	Paraffined glass	*Satisfied* (32° C.)	–	–	–	Rabbit, 5½	One single drop of blood placed on a *paraffined* glass slide under oil; end point when onset wrinkling of surface indicating beginning of clot formation	
D	1 Howell[58] (1914)	Oxalated plasma	Glass tubes	Considered	–	+	–	9-12	Optimal recalcification of oxalated centrifuged plasma; *end point* when tubes inverted without losing their contents	
	2. Gram[60] (1920)	Oxalated plasma	Glass tubes	Satisfied	–	+	–	3 6	Important modification of Howell's *technique*	
	3. Chantemesse[70] (1909)	Venous	Capillaries	----	–	+	–	—	Based on the point that specimens of good clot forming ability require a higher concentration of anticoagulant solution in order to prevent coagulation as compared to one with *poor clot* forming ability	Gelera[71] (1921)

B. Optical methods.
  1. Direct microscopy.
  2. Transillumination technique.
C. Van Allen's method.
D. Plasma coagulability tests.

On the basis of this classification a survey is given of the various methods in Table I with a brief indication of technique and points of principal interest. The survey does not pretend to represent a complete review of all published methods. Procedures are included only so far as a new principle or essential points of technical interest have been presented. Where a modification of a method has been worked out, this modification is included only in case of essential additions to the previously published method, and so indicated in the last column of the table. All other methods have been omitted. An historical review, however interesting and instructive it might be, has been left out. The year of publication of each method is included and chronologic order is maintained in the subgroups A subdivision into smaller units has been undertaken to facilitate the survey not only according to identity in principle, but also according to similarity of technique. By this arrangement it may also be possible to convey the impression how surprisingly few have been the leads offered the research worker interested in this problem.

Comment

It was previously stated that fundamental links are missing for a deeper understanding of the physiologic process of the coagulation of the blood. This is particularly felt when trying to present a constructive critical review of the methods listed in the table We are confronted here with the peculiarity of dealing with two partly separate problems, each of which is not completely understood, and for the explanation of which information regarding the one is derived from studies of the other.

In the subsequent exposition I have chosen to present my review in the form of a brief presentation of the more generally accepted observations bearing on the coagulation process, and further on the role of the platelets, particularly their importance for the inception of coagulation

As previously pointed out, coagulation of the blood in vitro is a result of the interaction of intrinsic hematologic and extrinsic factors. Without going into details regarding the physiology of this process, it may be stated that one of the more commonly accepted theories regarding this process is expressed briefly as follows:

1. Thrombokinase + calcium + prothrombin → thrombin
2. Thrombin + fibrinogen → fibrin.

Great interest has centered on the phenomena relative to inception of coagulation of the blood. A mass of experimental data has been presented with the object of elucidating the question whether the primary changes are to be found in the plasma or in the platelets. It is outside the scope of the present chapter to present a survey of these investigations. In addition, the

question appears still to be unsettled. However, the impression remains that the inception of blood coagulation in lower animals is primarily a process related to the cellular elements of the blood. In higher animals with the development of a complex plasma the role of the plasma may at least be equal to that of the platelets.[72-89]

Whichever point of view may be accepted, a protein-cephalin compound[90, 91, 92] appears to be the result of changes occurring in tissues, platelets, and the plasma complex, and normally of essential importance for the inception of coagulation. It is still unknown how such a disintegration may be brought about in vivo

When blood is spontaneously escaping from the organism, a protective mechanism aiming at an arrest of the hemorrhage is immediately switched into action. There is reason to believe that the inception of clotting under these conditions is similar to that observed under experimental conditions *and centered on the formation of thrombokinase. In this connection it makes* no difference whether the bleeding has occurred spontaneously or has been produced artificially, for instance, for investigative purposes (by pricking the lobe of the ear). Common is an external factor or factors breaking the stability of the blood, with subsequent onset of coagulation. Investigative data indicate that these factors may act through the platelets.

Bizzozero[93] wrote in 1882 "These platelets circulating in the blood can also be observed outside the circulation when the preparation is undertaken without delay. It is imperative to work fast, because the platelets are changed very rapidly and are not to be recognized after a few moments." When we, decades after Bizzozero's observations, are still heavily involved in discussions on the actual number of the blood platelets, it seems appropriate to recall his statement. What seems to be a peculiarity of the platelets of changing and disappearing under vision is in reality not a peculiarity but a *result of their functions, which may be listed as (1) coagulative, (2) adhesive* (3) agglutinative, and (4) retractive Because of these characteristics which render the platelets a very unstable constituent of the corpuscular elements of the blood, the platelets take their place as one of the important links in the protective mechanism active in the arrest of hemorrhage.

The corpuscular elements of the blood in circulation possess a certain suspension stability The maintenance of this stability is essential. It is considered to be regulated, at least partly, by the chemical and colloidal state of the plasma proteins[94-102] In vitro, occasionally also in vivo, changes occur in the equilibrium of this suspension stability Changes may be biologic as well as physical For instance, increased surface tension tends to produce agglutination while a counteraction is represented by the electronegative charge of the individual platelets

The agglutinating property of the erythrocytes is readily demonstrated by *the physiologic* phenomenon of rouleaux formation. As regards the platelets, a demonstration of agglutinability is more difficult to present, as agglutination and adhesion result in initial steps to disintegration of the platelets,

which thereby lose their morphologic characteristics. That disintegration has occurred can readily be demonstrated Platelet counts are found to be higher in a drop of blood obtained by venipuncture than in one obtained by capillary puncture, indicating the fallacy of the employment of capillary blood. By applying a series of drops on dry glass slides and deliberately delaying the making of the smears in some of the slides, one may find few or no platelets in the latter smears even when there is an abundance of platelets in the smears properly treated. These examples represent the negative side of the picture.

Adhesion of platelets can be observed grossly in the following manner: From a patient with a known high number of platelets that regularly occurs during the first two weeks after splenectomy, about 5 c.c. of whole blood is withdrawn into each of two regular test tubes that differ only in that one tube is given a fine coating of oil inside In the untreated tube I have repeatedly noted that the blood has given the glass wall a very fine, peculiarly uneven surface, reminding one of the coarse surface of a grinding stone. This appearance is the result of adherent and agglutinated small clumps of platelets. It is never demonstrable in cases in which the platelet count is normal or in the tube coated with oil.

Observations demonstrating the same properties of the platelets have been obtained by microscopic examinations of artificially produced lesions of capillaries and smaller vessels. These observations will be dealt with more closely in a subsequent chapter. However, mention may be made of the finding that platelets are retained by adhesion and agglutination adjacent to the vascular lesion or along its edges By initiating the clotting process and by mechanically closing the vascular lesion, the platelets may be considered of essential consequence for the duration of the bleeding Following a prick in the lobe of the ear, the bleeding is normally arrested after 1 to 3 minutes (bleeding time of Duke)

When using capillary blood for investigative purposes of the present nature, the depth of the prick, the size and form of the needle employed, the state of capillary filling, the pressure exerted on the tissues must all be considered of consequence for the wound surface lining the canal through which blood is reaching the surface Not less important is the degree to which tissue juices are mixed with the blood during this passage, when considering the highly coagulant effect of tissue juices Under these circumstances it appears practically impossible to obtain standardized experimental conditions These uncertainties can be dispensed with only by not employing capillary blood for studies of its coagulability

If the arrest of the hemorrhage from capillary bleeding is considered a progressive reaction, it is worth while remembering that when capillary blood is used for investigations we obtain a specimen which, so to speak, has been given a flying start by the agglutinated platelets adherent to the wound surfaces When this specimen is exposed to the surface of the glass tube or capillary tube, it is again receiving a second stimulus from disintegrating platelets on the glass surface The efficacy of each method depends upon the constancy

with which all these stimuli of external physical nature may be reproduced from observation to observation. Mention may be made of findings, indicating that there exists a certain relation between the coagulation on the one hand and the volume of the specimen of blood in particular relation to the amount of its surface exposed to the glass on the other hand. Wöhlisch[34] made a careful investigation on this point and stressed the necessity of always working with eyeglasses of a constant diopter and drops of blood of constant volume in order to assure comparable results. He furthermore emphasized the necessity of avoiding mechanical stirring of the specimen, as this hastens the clotting.

It may be opportune at this time to bind together the various parts of this statement in a brief summary:

The inception of coagulation of the blood in vitro is a process related to fundamental changes in the platelets and the plasma complex. The coagulant activity of the platelets is liberated by their disintegration, a result of their agglutinative and adhesive properties. This introduces the physical factor as of essential importance for the process of blood coagulation. We do not at present know how the plasma is directly affected by physical factors. We definitely know that platelets during exposure to these factors (surfaces of wound, metal and glass material) are disintegrating, and thereby to a definite extent govern the velocity of coagulation. If coagulation is considered as a chemical process, it follows that the velocity of this reaction is closely related to a physical factor of a different order; namely, the temperature.

I have intended with this exposition to present vital biologic problems related to the coagulation of the blood in an admittedly simplified form and of a sequence naturally leading to a realization of the consequences of physical factors for the present problem. The hematologic factors through which these factors are primarily exerting their influence are represented by the platelets, particularly through their agglutinating and adhesive properties. It is hoped that the evidence presented in this chapter may justify and substantiate the definition of the coagulation time as presented in the previous chapter.

Besides having worked out the hematologic factor through which the physical factors exert their influence, a good many investigations have been undertaken in order to learn the nature of these external influences. A most valuable contribution to this problem has been presented recently by Lampert,[44, 45] whose work is a direct continuation of problems which had occupied hematologists for decades. For a long time it has been well known that blood received in an oily or paraffined test tube exhibits a retardation of coagulation as compared to the sample contained in an untreated test tube. Bordet and Gengou were of the belief that this was due to the fact that paraffin was not "wettable" by blood.

Lampert devised a method by which he could obtain a measurement of the surface tension of blood in relation to various materials of which containers were made. By estimating the coagulation time of blood deposited in test tubes of different material, he was able to show that a parallel existed be-

tween the coagulation time and the surface tension of the blood in relation to the particular container. On the basis of this finding Lampert searched for suitable materials and found in *bernstein* and *athrombit* (artificial resin products) material for test tubes in which the relative surface tension of the blood was approaching that contained in a paraffined test tube. With athrombit was noted a coagulation time approximately that observed in paraffined test tubes. The chemical composition of the glassware was found of consequence in that glass of a poor quality, containing relatively much alkali, produced a hastening of the coagulation time.

Lampert drew the practical conclusions of these investigations by the construction of various hematologic utensils made of athrombit (apparatus for determination of the coagulability of the blood, a pipette for counting platelets, containers for direct transfusion of blood, special syringes, and so forth). His apparatus for study of the coagulability of the blood will not be described here; it is a modification of the method of Heubner and Rona. The normal coagulation time of the blood of man is about 30 minutes at 37° C., according to the technique of Lampert.

As glassware is probably the most commonly employed material in construction of laboratory utensils for hematologic investigations, the practical consequence of Lampert's work is obvious. In this connection it may be mentioned that several other investigators prior to Lampert have also examined carefully the effect of other material Stern[103] (1916), using the viscosimeter of Cannon and Mendenhall, found a shortening of the coagulation time by using an iron or an aluminum wire instead of copper. Stern also investigated the physical factor represented by a current of electricity and found an increasing shortening of the coagulation time with an increase of the electric current. Stern does not present any suggestions as to the mechanism of these physical factors Lampert stated that the surfaces of the vessels exert a mechanical catalytic action on the coagulation Lampert, however, does not follow up this interesting statement by more than a suggestion that this effect is produced through the platelets It is my impression that a problem of no small theoretic consequence is left unanswered here by Lampert The feeling remains that Lampert's setup may permit studies of changes in the suspension stability of the platelets (electronegative charge) in various milieus (glass, athrombit) in direct relation to the inception of blood coagulation

Takashima,[104] in a later publication (1934), has reported similar observations. His findings are a substantiation of Lampert's work The coagulation time measured according to the technique of Takenaka (coaguloviscosimeter) was found to be shortest in vessels where the disintegration of platelets was found to be most marked. The latter was ascertained by counting the platelets.

Leiri[105] (1934) has considered the coagulation of blood mainly on a physical basis No experimental data are presented in his article and the reader obtains the impression that the publication is meant as a presentation of a working hypothesis As such, it appears most suggestive. As it may be considered a step beyond Lampert's work, a short outline of its main points is included here.

According to Leiri, the colloidal particles of the blood possess an electronegative charge, while the dispersion fluid carries an electropositive one. While blood is in circulation the electropositive charge of the dispersion fluid is relatively increased, while no greater physical change occurs in the colloidal particles. This assures a stability of the fibrinogen, which is the protein most susceptible to physical changes because its iso-electric point approaches the pH of the blood (iso-electric point of albumin, globulin, and fibrinogen is measured at pH 4.7, 5.4, and 7.0, respectively [Michaelis[106]]). In stagnated blood this relative difference between the two opposite electric charges is diminished, thereby facilitating precipitation of fibrinogen.

When blood is placed in a receptacle of one type or another, a double electric layer is formed between the blood and the wall of the vessel. If this receptacle is made of glass, its wall is electronegative in relation to the electropositive charge of the blood. The electropositive ions of the blood are attracted to the negative ones of the glass wall, thereby rapidly reducing the original difference in electric charge

A somewhat different condition is encountered if blood is placed in receptacles made of gold, other precious metals, or inoxidizable steel. The electropositive ions of the wall of the container in these cases do not pass into the liquid. The wall maintains its electropositive charge in relation to the electropositive charge of the blood, and the blood retains its fluid state for a considerable time

Leiri maintains that the velocity of ion exchange during various conditions is affected by the temperature, thereby intimating that this physical factor may influence the velocity of blood coagulation not only through chemical factors alone, as mentioned previously, but possibly also through purely physical influences as well.

The setting of the problems as presented by the publication of Leiri is not new. Fåhraeus[94, 95, 96] has dealt with similar physical problems It is likewise considered by Starlinger[102] in extensive investigations. This latter work will not be considered here, as it is concerned with the direct applicability of these points of view to disease and not to the physical aspects of blood coagulation

Let us now, with these physical aspects of the problem in mind, return to a further discussion of technique It will be readily understood that, when a specimen under investigation is brought into movement by direct stirring, by tilting of the tube, by gravity, or by positive pressure arrangements, an uncontrollable physical factor is thereby introduced Practically all the methods based on viscosimetry in one form or another have to deal with this factor, not least the methods employing direct viscosimetry. In the description of technique various authors have consequently felt the necessity of going into minute details regarding this part of the procedure Most elaborate in this respect is the description by Bürker. Others have only indicated the tilting or stirring technique, with approximate intimation of the time intervals for this procedure. Common for most of the methods is the fact that this mechanical procedure cannot be dispensed with without robbing the methods of their end

points. This is in sharp contrast to most of the optical methods where an absolute requirement for the successful optical reading is that the specimen remains completely at rest during the observation.

Uncontrollable physical influences may be incidentally introduced by working with laboratory utensils, syringes, pipettes, and capillary tubes which are not completely dry. If they are still wet, the surface action of the material in relation to the blood will be changed. Water itself also hastens the coagulation, an effect possibly of chemical nature. A reservation to the requirements stated here must be made in relation to procedures where the use of utensils with dry surfaces has been discontinued in order to be able to use syringes with an inner protective coating, for instance, of oil or containing an anticoagulant solution.

In connection with this discussion it may be opportune to return to Lampert's work Can it be said that his studies have formed a basis for ideal methods for determination of the coagulability of the blood?

By using laboratory implements of the type employed by Lampert for the drawing and reception of blood, many of the inconsistencies and errors of the present-day technique may be avoided. A closer investigation of this type of utensil had been planned in the present work, but on communicating with the firm that manufactured the Lampert apparatus for blood transfusions, we were informed that the production of the more commonly used laboratory implements of athrombit had been discontinued

The long normal coagulation time with the universal thrombometer of Lampert, however, in itself, cannot indicate any efficacy and dependability as an instrument for determination of the coagulability of the blood. The prolonged time indicates that an external factor of reduced magnitude is governing the velocity of the reaction. According to the criteria previously stated for an ideal method, the important point is that the stimulus of the external physical factors remains constant, or approximately so, from observation to observation With our present information we do not definitely know whether this is accomplished more easily with a weak stimulus or with a more marked one. At this time, however, it appears that the coagulation time per se cannot be considered an indication of the efficacy of any method for the measurement of the coagulability of the blood

Methods Concerning the Coagulability of the Blood Plasma

The methods dealing with investigations of the coagulability of the blood plasma will be considered separately, as they form the basis of the present study

For clinical determination of the coagulability of the blood, the untreated whole blood generally has been used most frequently up to this time Due to its relatively rapid and progressive changes following withdrawal, the untreated whole blood has been less applicable to experimental investigations concerning the physiology of the process of coagulation, where a fundamental requirement of the study is that the identical specimen may be observed under

various experimental conditions. For the latter investigations various types of blood plasma have been employed extensively in the different physiologic laboratories for many years. From the very beginning these investigations have been associated with the role of calcium in the mechanism of the coagulation process.

These particular researches date back to the latter part of the preceding century. Green[107] (1887), during studies of the coagulation of magnesium sulfate plasma by the addition of fibrin ferment, found by quantitative analysis that the fibrin ferment obtained by salt extraction of washed blood clots contained a definite and fairly constant amount of calcium sulfate. By adding a saturated solution of calcium sulfate to diluted magnesium sulfate plasma, rapid coagulation occurred. Green suggested that the fibrin ferment was active only through the presence of its inorganic ally. Only three years later Arthus and Pagès,[108] through their investigations of the coagulability of milk, prevented coagulation of the blood in a manner identical to that of milk by the addition of alkali oxalate. They succeeded further in demonstrating that this oxalate plasma is rendered coagulable by the addition of a definite concentration of calcium chloride solution From their investigations Arthus and Pagès concluded that alkali oxalates exert their anticoagulant action through a precipitation of calcium *("Les agents décalcifiant sont des agents anticoagulants")*. Their further conclusion was that soluble calcium is necessary for coagulation of the blood *("La présence des sels de calcium dissous dans le sang est une condition nécessaire de la coagulation."*—Arthus[109]).

Pekelharing[110] (1892) likewise explained the anticoagulant action of alkali citrate on a similar calcium inactivating basis

Since the appearance of these original contributions, much investigative work has been performed regarding the more intimate mechanism of calcium in the coagulation of the blood. Much controversy has arisen on this point, particularly since finding that an excess of oxalate is necessary in order to prevent coagulation Collingwood[112, 113] and Vines[111] considered the previous explanation of the activity of calcium as erroneous. Stuber[114, 115] and his school denied calcium any essential role in this process and explained the anticoagulant action of oxalates and citrates by the formation of a maximally ionized salt-fibrinogen complex which, by dilution or further addition of salt, is rendered coagulable through a reduction of ionization of this complex According to later research the importance of such a complex must be considered refuted. By a special procedure Nordbo[116] succeeded in removing the calcium from the blood gradually without the addition of anticoagulants or other calcium-binding substances The clot-forming ability of the specimen was reproduced by the addition of the calcium equivalent to the original calcium content of the blood. The necessity of the addition of alkali oxalate in excess of the available, chem[illegible]
cification [illegible]
lent quan[illegible]

ever, is a protracted process which is not ended before the coagulation is completed (Nordbö). This relative time factor is counteracted by an excess of oxalate.

The original conception of Arthus and Pagès and Pekelharing must be considered substantiated.[118-119] *Chemically active ionized calcium is necessary for the coagulation of blood. Alkali oxalate or citrate exerts its anticoagulant action through a precipitation of calcium as calcium oxalate or through a binding of calcium as insoluble calcium citrate. The restitution of coagulation in an oxalated or citrated blood specimen by recalcification is the result of substitution of soluble calcium ions.*

In the field of clinical medicine the practicability of blood plasma as an investigative basis appears to have received relatively little attention as compared to the numerous methods dealing with untreated whole blood.

Chantemesse[70] (1909) presented his technique for a clinical test based on the finding that blood of varying coagulability requires a varying minimal concentration of anticoagulant solution for the prevention of coagulation. A minute volume of blood was mixed with an identical volume of a solution of potassium oxalate of concentrations increasing from 1/1,800 to 1/400. The series of blood-oxalate mixtures was placed in capillary tubes. At the end of one hour the tubes were inspected and the concentration of oxalate preventing coagulation was noted. Considering this minimal concentration in a normal case to be 1/600 oxalate solution, a specimen of blood requiring a stronger solution for prevention of coagulation was considered to have coagulating ability better than normal, while a sample requiring a weaker solution was said to exhibit less marked coagulating ability.

This test of Chantemesse does not represent exactly what we mean today when talking about tests for coagulability of the plasma. As will be understood, there is no essential difference whether we use oxalated whole blood or plasma for our investigations, as will be later indicated. His technique is also of interest from another point. While all subsequent methods of this type employ recalcification, Chantemesse used a simplified procedure consisting in reality of only the first half of recalcification observation. The same line of reasoning is the basis for a test published by Gelera in 1921[71] It cannot be seen that any of these methods has been clinically applied to any further extent.

Howell in 1914[49] reported his investigations on oxalated plasma by recalcification with an optimum concentration of calcium chloride. According to his technique, he found a remarkably uniform coagulation time of recalcified plasma in normal persons varying between 9 and 12 minutes Very marked prolongation was noted in cases of hemophilia, an observation also made in 1911, by Addis,[120] who did not appear to have attached any particular significance to the finding. For theoretic reasons Howell at the time considered the clotting deficiency in hemophilic blood due to a deficiency of prothrombin. The recalcification of oxalated plasma was considered by Howell, therefore, a test for detection of prothrombin deficiencies, and subsequently came to be named Howell's test for determination of prothrombin time This terminology was rather unfortunate Combined with the fact that the work was presented

by an authority like Howell, it may have contributed to sidetracking the practical applicability of recalcified plasma, at least in the American laboratories

In 1926 Howell and Cekada[121] again took up the problem of deficiencies of hemophilic blood and concluded, according to renewed investigations, that prothrombin in blood of hemophiliacs did not differ from that in normal blood, either in its concentration or in its properties.

This finding did not change Howell's original observation. The theoretic basis for his original interpretation of his observation, however, could not be maintained. Unfortunately the technique employed by Howell in 1914 is still described in hematologic textbooks as determining the prothrombin time. This has given rise to misunderstandings.

In the meantime Gram[89] in 1920 had begun to revise Howell's technique, particularly in regard to temperature requirements. Gram found a moderate prolongation of the coagulation time of recalcified plasma in cases with thrombocytopenic purpura, as well as a marked prolongation in three cases of hemophilia. He also undertook a comparison with other tests (capillary methods and Wright's test) and found less satisfactory results with these than with those obtained with recalcified plasma. Everything indicates that Gram did not look upon the test in the sense of Howell's conception, as he stated that the method was superior to the other more generally employed coagulation tests. In subsequent literature on the subject Gram's interesting report does not appear to have been given due attention.

Smith[122] (1925) obtained plasma by adding a solution of sodium chloride to the blood and inducing coagulation by the addition of a certain amount of distilled water or a solution of calcium chloride. The observations were made at room temperature and the end point was determined by inverting the tubes. Smith noted prolongation of the coagulation time of blood plasma in certain cases of jaundice, but he could not correlate this with the clinical evidence of hemorrhage in jaundice.

Fischer[123] in 1930, in connection with a description of a technical arrangement, reported satisfactory results by the use of salt plasma to which were added solutions of tissue thrombokinase or other solutions, the coagulative strength of which was to be examined. His investigations were directed more to analytic physiologic problems regarding the process of coagulation.

In 1929 Bancroft, Kugelmass, and Stanley-Brown[124] published their first results in a series of reports dealing with the evaluation of clotting factors in surgical disease. On the basis of theoretic considerations, with particular reference to Howell's theory, these investigators worked out a "clotting index." This was presented in the form of an equation arrived at "by placing the factors tending toward clotting, i e., prothrombin and fibrinogen as the numerator, and antithrombin which deters clotting as the denominator."

$$\text{Clotting index} = \frac{\text{Fibrinogen per cent by prothrombin index}}{\text{Antithrombin index}}$$

Considering the normal fibrinogen to be 0.5 to 0.7 per cent, the normal prothrombin and antithrombin index to be 1.0, the clotting index of normal

subjects was found to be 0.5 to 0.7. The prothrombin index was found by dividing the prothrombin time of a patient with normal prothrombin time, while the antithrombin index was determined by dividing the normal antithrombin time by that of a patient. A higher clotting index was considered indicative of a tendency to thrombosis; a lower one indicated a tendency to bleeding.

In reviewing the basis of this work it is well to remember that Howell's theory of blood coagulation has so far proved to be one of the most stimulating working hypotheses on the subject. The difficulty arises when an attempt is made to transform this theory into a practical formula for the determination of coagulability. Further obstacles are encountered when searching for adequate methods for the practical determination of each factor of the equation. *Following Howell and Cekada's report of 1926, Howell's original test for prothrombin* time cannot be employed in the equation as basis for the determination of the prothrombin index as done by Bancroft and his associates. As will be touched upon later in this work there is, furthermore, no definite relation between the fibrinogen content of the blood and its coagulability.

In spite of the obviously questionable theoretic basis of the work of Bancroft and his associates, certain relations were reported between the hematologic observations and various clinical conditions (hemorrhagic diathesis, postoperative thrombosis). Lewisohn[135] reported similar results with the same technique.

In order to arrive at an impression of the practical applicability of the conception presented by Bancroft and his associates, in 1932 I undertook similar investigations on a large amount of clinical material.[126-128]

Among other points it was found that the coagulation time of the recalcified plasma depended on the length and speed of centrifugation of the plasma, making it most unlikely that the test was to be considered an expression of the quantity of prothrombin present In a few cases in which there was clinical evidence of a hemorrhagic diathesis, it was noted that the clotting index, computed according to the equation mentioned previously, was normal, due to increased fibrinogen in spite of prolongation of the coagulation time of the recalcified plasma. A good parallelism was noted between the clinical condition of hemorrhagic diathesis (thrombocytopenic purpura, hemophilia, cholemic hemorrhages) and the coagulation time of the recalcified plasma, as well as between the latter and the commonly used coagulation test of Lee and White.[37] Our conclusion was that *the coagulation time of recalcified plasma simply determined the coagulability of the plasma.* Considering further the plasma with the platelets as representing the essential coagulant constituents of the blood, *it was inferred that recalcification of the plasma according to a standardized technique represents a method for determination of the general coagulability of the blood.* It was termed the plasma coagulability test. As such it has been later employed by me in my investigations

It ought to be stated here that these conclusions were nothing more than an essential substantiation of the conclusions which may be inferred from the report of Gram,[69] whose work unfortunately had been overlooked at the time of our first report.

In a later report Bancroft and his associates[130] revised their original work according to similar views as presented above (1935). With the cooperation of Quick this problem was carried further by devising a test which gives an impression of the presence or absence of a prothrombin deficiency. This test will be considered in detail in a subsequent chapter.

Extensive studies on the coagulability of the blood plasma have also been undertaken by Dyckerhoff and his associates.[131] These investigators worked with oxalate or ammonium plasma. Immediately after withdrawal, the blood was centrifuged and the plasma was pipetted off and placed in the refrigerator at 2° C. At different intervals the plasma was taken out and warmed to 37° C., following which 1 c c. of plasma was recalcified with 0 3 c.c. of an optimal solution of calcium chloride. The normal coagulation time of plasma treated in this manner was found to be 1 minute and 40 seconds at 37° C. At 21° C. it was 4 minutes. To increase the sensitivity of this test, Dyckerhoff and his associates diluted the plasma with distilled water or saline solution and recalcified it with a 1 per cent solution of calcium chloride. By constant and gentle shaking of the tube one could easily observe by the naked eye the formation of fine fibrin threads. Dyckerhoff found no change in the coagulation time during the first two days after withdrawal of the blood. After this the coagulation time increased moderately until it was about 12 minutes at the end of a week.

Kürten and Harzer[132] have taken up this technique. A practically identical coagulation time of the recalcified oxalated plasma was noted in normal subjects as well as in patients with various diseases, according to this technique. On the basis of the work of Dyckerhoff and his associates as well as on that of their own, Kurten and Harzer introduced the expression "*Konstanz des Blutgerrinungssystems,*" and intimated that the prolongation of the coagulation time as observed by other investigations may be caused primarily not by a change in the clotting factors, but as a result of other influences from the metabolic process (liver, bile, nutrition, and so forth)

Optical Methods

It seems desirable to consider more closely the methods employing optical technique in one form or another. By these means an interesting perspective of the present problem may be obtained.

Although Brodie and Russell[15] made use of a microscope for reading the progress of coagulation, it will not be included among the optical methods. Their optical reading technique is aimed primarily at a more accurate estimation of the arrest of movements of the erythrocytes in a hanging drop of blood exposed to intermittent mechanical influence by a gentle flow of air. It seems natural, therefore, to place this method among those based on viscosimetry.

The optical procedures to be considered in this chapter are concerned primarily with a direct or indirect optical registration of the fibrin web and its further consolidation.

The first and simplest of the optical methods is that presented by Schwab[40] (1906). A hanging drop of blood is observed under oil immersion magnification. In the outer circumference of the drop comparatively few erythrocytes may be observed in an otherwise essentially clear plasma layer which is, therefore, chosen for observation. The interval from the taking of the blood to the first appearance of the characteristic fibrin web is considered the coagulation time. This was found to be at an average of 5 minutes and 30 seconds in normal subjects. To judge from the literature, this method has not been adopted for extensive clinical investigation, possibly because of the difficulty connected with satisfactory temperature arrangements. This technical obstacle appears to have been overcome by Fuchs[11] (1930).

With improving microscopic technique a series of interesting studies related to blood coagulation and its morphology was undertaken with the ultramicroscope by Mayer[133] (1907), Cesana[134] (1908), Stübel[135, 136] (1914), and Howell[137] (1914). These studies were primarily of theoretic interest. Kitamura[138] (1924) carried these investigations further. He made use of centrifugalized oxalated plasma which coagulated by dilution or by addition of calcium chloride. On the basis of morphologic differences, Kitamura divided the process of fibrin formation into five different types, which I shall not consider here. His time observations are of interest in this connection, however. In plasma from a rabbit he noted the first appearance of fibrin needles after 9 minutes, a well-formed fibrin web at 14 minutes, and completed coagulation after 23 minutes. Kitamura suggests that his technique is applicable to clinical investigation, a suggestion which does not appear to have been taken up by the clinical laboratories to any extent as yet. The importance of these ultramicroscopic investigations is to be found in their clear demonstration that there is a free interval during coagulation before occurrence of the fibrin; further, that after fibrin needles are formed a certain period is required before the fibrin web is completed and coagulation ended

The further steps in the development of optical methods are instructive. They clearly illustrate the close interrelation of progress in various branches of the natural sciences.

With the ever-widening scope of colloidal chemistry during the first two or three decades of this century, the parallel progress of technical devices for the registration of physical phenomena was rapidly encouraging further research The dispersion of colloidal systems was studied by the aid of optical methods registering the change in transparency under various experimental conditions by electrical units of one type or another

Kugelmass[39] (1922) applied this type of investigation to the study of the process of coagulation of blood He made use of an apparatus termed the nephelometer This consisted of a light bulb furnishing constant illumination of the specimen under observation The latter was placed between the source of light and a thermophile connected with a sensitive galvanometer During coagulation a variation occurred in the transillumination. This was registered by the thermophile as a variation in radiant heat energy reaching this instru-

ment. By reading the movements of the galvanometer needle, these values, when plotted on paper, indicated the coagulation curve, as illustrated in Fig. 1.

For his investigations Kugelmass made use of oxalated plasma or solutions of fibrinogen brought to coagulation by the addition of a solution of thrombin, which formed part of the basis for his important studies of the physiology of the coagulation of the blood. It appears that he has not made use of this device in his later clinical research.

In an article by Festen[141] casual mention is made of investigations by Wolvius[139] (1923), apparently on a basis resembling that of Kugelmass. The author has not succeeded in obtaining additional information regarding this work.

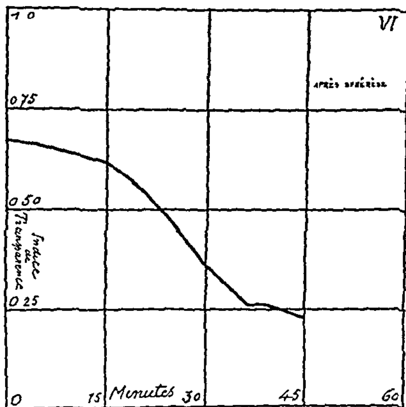


Fig. 1.—Plotted curve of the coagulation of recalcified plasma based on the use of the nephelometer. (After Kugelmass.)

Klinke and Elias[62-63] in 1931 reported a series of investigations of blood coagulation. These men, like Kugelmass, directly profited from previously employed colloidal technique Considering blood plasma a colloidal system, they reasoned that any change in the dispersion of this system resulting from the transition of fibrinogen to fibrin would lead to a variation in the Tyndall effect. This latter effect was studied by a Zeiss-Stufen photometer For technical reasons the use of whole blood was ruled out Instead they resorted to the use of citrated plasma brought to coagulation by recalcification. Readings of the

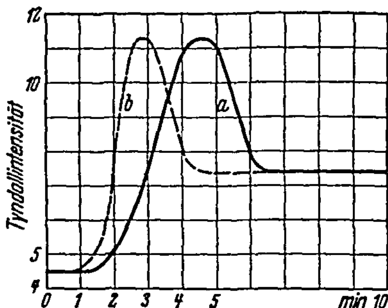


Fig 2.—Plotted curve indicating the variation of the Tyndall effect during coagulation of recalcified citrated plasma. (After Klinke and Elias.)

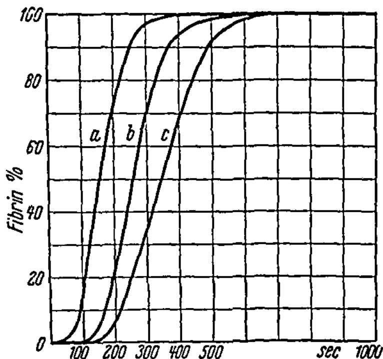


Fig 3.—Mathematically computed and corrected curves of the variation of the Tyndall effect during coagulation of recalcified citrated plasma (After Klinke and Elias.)

Stufen photometer were made every 15 to 30 seconds. The readings, when plotted on paper, gave a curve directly indicating the variation of the Tyndall effect during the process (Figs. 2 and 3.) Because of reduction of transmitted light during the reaction and due to peculiarities of the colloidal system under investigation, the curve had to be corrected by intricate theoretic considerations and mathematical computations. The corrected curve appears similar to that obtained, for example, by Takenaka's[58] direct viscosimetric determinations (Fig. 4) as well as by Kugelmass. From the velocity of the reaction as indicated *by the corrected curves, Klinke and Elias concluded that the transition* of fibrinogen into fibrin is a fermentative process. The technical procedure as employed by these workers is not directly recommended for clinical investigations, although their reports include a definitely pathologic coagulation curve of a patient with hemophilia.

In 1936 Baldes and I[140] reported investigations of the coagulation of blood plasma, using the photo-electric technique. This is the basis of the present work. It is only fair in this connection to point out that rapid development of the theoretic and practical aspects of photo-electricity during the last years has encouraged this type of hematologic research, together with numerous other types of studies of direct medical interest to be brought out in a subsequent chapter. At the time of Kugelmass' interesting investigations, the reliability of the photo-electric measuring devices was not such as to form the basis of more accurate investigations. His experimental setup could hardly have prevented him from working out the subsequent photo-electric investigations if satisfactory instruments had been available at that time

It may be mentioned, finally, that Festen in 1937[141] reported a method for photo-electric examination of the coagulability of whole blood.

Summary

It is stressed that the fluidity of the blood must be considered a problem different from that of its coagulation.

The ability of the blood to be transformed from the fluid to the clotted state must be considered mainly a potential property It is generally an in vitro phenomenon *Coagulation is considered as the resultant activity of factors of hematologic as well as of purely nonhematologic extrinsic nature.*

In mammals the extrinsic factors (wettable surfaces like glass and metals, chemicals like calcium and thromboplastin) initiate the process of coagulation through breaking or disturbing the stability of the blood plasma and the blood platelets.

The velocity with which the blood in vitro is transformed from the fluid to the clotted stage depends, on the one hand, upon the coagulant activity of the intrinsic hematologic factors, and on the other hand, upon the extrinsic, nonhematologic factors. By keeping the latter factors constant, the changing velocity of the process represents a relative expression of the coagulant factors of hematologic origin. This is the basis of any method dealing with measurements of the coagulability of the blood.

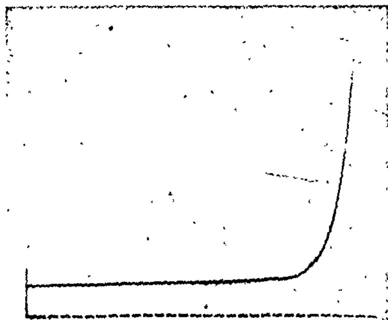


Fig. 4.—*Tracing of the coagulation of whole blood obtained by Takenaka's viscosimeter.* (After Takenaka.)

The main requisite for any dependable method is that the external stimulus initiating the reaction can be kept constant from one observation to another. Dependability is not assured by diminishing this stimulus. The dependability of any procedure is therefore not measurable on the basis of its "normal coagulation time"; this dependability can be decided upon only after due consideration of its merits when applied to clinical and experimental investigations. It will also be understood that it is irrelevant to talk of a "physiologic coagulation time" of the blood. Any "normal coagulation time" must be considered normal only in relation to the applied technique.

The dependability of a method is not increased by its ability to register both the beginning and the end of the process of coagulation. From an investigative point of view such a method may present definite advantages. From a practical point of view it does not add to its clinical applicability. As will be shown later, there is a constant relation between the first and the second stage of the process; furthermore, the latter continues past the formation of a clot.

A survey is presented of the methods concerning measurements of the coagulability of the blood, with a brief indication of the essential characteristics of each method. A brief consideration of the various readings of the end points of the process is presented. It was found that the development had been centered largely on the improvement of reading technique, while other important factors may not have been duly considered This may partly explain less satisfactory results and controversial issues in spite of a large accumulation of data.

References

1. Fonio, A.: Die Gerinnung des Blutes. In Bethe, A, von Bergmann, G, Embden, G., and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie mit Berücksichtigung der experimentellen Pharmakologie, Berlin, 1928, Julius Springer, vol. 6, pp 307-400
2. Berger, Wilhelm, and Petschacher, Ludwig Vergleichende Untersuchungen zur Mikro-Eiweissanalyse des Blutserums, Ztschr f d. ges. exper. Med. 36. 258-296, 1923.
3. Nygaard, K. K., Wilder, Marion, and Berkson, Joseph The Relation Between Viscosity of the Blood and the Relative Volume of Erythrocytes (Hematocrit Value), Am. J. Physiol. 114: 128 131, 1935.
4. Vierordt: Quoted by Fonio, A.[1]
5 Kottmann, K., and Lidsky, A Die Vierordtsche Methode für Gerinnungsbestimmungen des Blutes in verbesserter Form, Ztschr. f. klin. Med. 69: 431-434, 1910.
6 Bürker, K.: Ein Apparat zur Ermittelung der Blutgerinnungszeit, Arch. f. d. ges. Physiol. 118. 452-459, 1907
7 Bürker, K Vereinfachte Methode zur Bestimmung der Blutgerinnungszeit, Ibid. 149 318-326, 1913.
8 Perrin, Maurice, and Hanns, Alfred Méthode pratique d'appréciation du début macroscopique de la coagulation du sang, Compt. rend. Soc. de biol. 87: 1215-1216, 1922
9 Kaufmann, E.: Ein Blutkoagulotachymeter, Klin Wchnschr 4: 1383, 1925.
10. Sabrazès, J.: Procédés pratiques pour déterminer, au lit du malade, le début de la coagulation du sang et pour faciliter l'examen du caillot et du sérum, Folia haemat. 1: 394-396, 1904.
11. McGowan, J. P.: A Clinical Method for Estimating the Coagulation Time of the Blood, Brit. M. J. 2: 1580 1581, 1907.

12. Addis, T.: The Ineffectiveness of Calcium Salts and of Citric Acid as Used to Modify the Coagulation Time of the Blood for Therapeutic Purposes. With a Description of a Modification of McGowan's Method of Estimating the Coagulation Time of the Blood, Brit. M. J. 1: 997-999, 1909.
13. Petersen, M. F., and Mills, C. A.: A New Method for Accurately Determining the Clotting Time of the Blood, Arch. Int. Med. 32: 188-191, 1923.
14. Schultz, Werner: Eine neue Methode zur Bestimmung der Gerinnungsfähigkeit des Blutes, Berl. klin. Wchnschr. 1: 527-528, 1910.
15. Brodie, T. G., and Russell, A. E.: The Determination of the Coagulation Time of Blood, J. Physiol. 21: 403-407, 1897.
16. Boggs, T. R.: Some Clinical Aspects of Blood Coagulation, Internat. Clin. 1: 31 39, 1908.
17. Fuchs, H. J.: Ein elektrischer Heiztisch und ein einfacher Apparat zur Bestimmung der Blutgerinnungszeit, Ztschr. f. Immunitatsforsch. u. exper. Therap. 69: 58-62, 1930.
18. Buckmaster, G. A.: Quoted by Cohen, M. S.[31]
19. Inchley, O.: A Simple Method for the Determination of the Coagulation Time of Blood in Animals, J. Pharmacol. & Exper. Therap. 18: 237-240, 1921.
20. Gibbs, O. S.: A Clinical Blood Coagulometer, Quart. J. Med. 17: 312-318, 1924.
21. Gibbs, O. S.: On Measurement of Blood-Coagulation Time, J. Physiol. 59: 426-433, 1925.
22. Biffi, U.: Quoted by Cohen, M. S.[31]
23. Fuld, E., and Schlesinger, Erich: Ueber die Gerinnung des Blutes, Berl. klin Wchnschr 2: 1323-1327, 1912.
24. Hedenius, P.: An Apparatus for Determining the Coagulation Time of the Blood, Acta med. Scandinav. 88: 440-442, 1936.
25. Szankowski, Wlodzimierz· Über eine neue Methode der Bestimmung der Gerinnungszeit von Blut und Plasma, Arch. f. exper. Path. u. Pharmakol. 184: 317-326, 1937.
26. Hayem, G.· Du caillot non rétractile. suppression de la formation du sérum sanguin dans quelques états pathologiques, Compt. rend. Acad. d. sc. 123: 894 896, 1896.
27. Lee, R. I., and White, P. D.: A Clinical Study of the Coagulation Time of Blood, Am. J. M. Sc. 145: 495 503, 1913.
28. Milian, M. G.. Quoted by Schultz, W.: Die Untersuchungsmethoden der Blutgerinnung und der Blutgefassfunktion in ihrer Beziehung zur Blutstillungsfrage, Handbuch allg. Haematol. 2: 645-700, 1933.
29. Hinman, Frank, and Sladen, F. J : Measurement of the Coagulation Time of the Blood, and Its Application, Bull Johns Hopkins Hosp. 18: 207-220, 1907.
30. Duke, W. W.: The Relation of Blood Platelets to Hemorrhagic Disease; Description of a Method for Determining the Bleeding Time and Coagulation Time and Report of Three Cases of Hemorrhagic Disease Relieved by Transfusion, J. A. M. A 55: 1185-1192, 1910
31. Cohen, M. S.: The Coagulation Time of the Blood as Affected by Various Conditions, Arch. Int. Med. 8: 684-716, 820-850, 1911.
32. Pétren, Gustav: Untersuchungen uber die Blutgerinnung bei Icterus, Beitr. z. klin. Chir. 120: 501-588, 1920.
33. Morawitz, P., and Bierich, R.: Über die Pathogenese der cholämischen Blutungen, Arch. f. exper. Path. u Pharmakol 56: 115-129, 1907.
34. Wohlisch, Edgar: Die physikalischen Grundlagen einer rationellen Methodik zur Bestimmung der Gerinnungszeit des Venenblutes (Untersuchungen uber Blutgerinnung IV), Ztschr. f. d. ges. exper. Med. 27: 61-81, 1922.
35. Wright, A. E.. On a Method of Determining the Condition of Blood Coagulability for Clinical and Experimental Purposes, and on the Effect of the Administration of Calcium Salts in Haemophilia and Actual or Threatened Haemorrhage, Brit. M. J. 2: 223-225, 1893

36. Yatsushiro, T.: Experimentelle Untersuchungen über die Thrombosenfrage, nebst Angabe einer einfachen Methode zur Koagulationsbestimmung des Blutes, Deutsche Ztschr. f. Chir. 125: 559-612, 1913.
37. Mas y Magro: Studien über Blutgerinnung. I. Bestimmung der Gerinnungszeit, Arch. cardiol. y hemat. 5: 288-294, 1924; abst. Ber. ges. Physiol. 29: 806, 1925.
38. Prawdicz-Neminski, W. W.: Anschauliche Methode der fraktionierten Blutgerinnungsbestimmung, Ztschr. f. d. ges. exper. Med. 54: 820-825, 1927.
39. Heubner, W., and Rona, P.: Methode zur Bestimmung der Blutgerinnungszeit, Biochem. Ztschr. 130: 463-475, 1922.
40. Frisch, A., and Starlinger, W.: Zur Methodik der Bestimmung der Blutgerinnungszeit, Wien. klin. Wchnschr. 34: 344-345, 1921.
41. Homma, Hans: Ein neuer Apparat zur Bestimmung der Blutgerinnungszeit, Ibid. 43: 1474-1475, 1930.
42. Trought, H., and Riddoch, J. W.: Estimation of the Coagulation Time of the Blood: a Simplified Method, Brit. M. J. 2: 1194-1195, 1936.
43. Van Walsem, G. C.: Mikroverfahren zur Bestimmung der Koagulationsgeschwindigkeit des Blutes, Nederl. tijdschr. v. geneesk. 2: 4944, 1928; abst. Ber. ges. Physiol. 48: 396, 1929.
44. Lampert, Heinrich: Die Bestimmung der Blutgerinnungszeit, München. med. Wchnschr. 77: 586-588, 1930.
45. Lampert, Heinrich: Die physikalische Seite des Blutgerinnungsproblems und ihre praktische Bedeutung, Leipzig, 1931, Georg Thieme, 127 pages.
46. Belák, Alexander: Eine neue Methode zur Bestimmung der Gerinnungszeit des Blutes, Deutsche med. Wchnschr. 1: 433-434, 1919.
47. Geers, J.: Ein Versuch die Gerinnungszeit des Blutes genau zu messen, Nederl. tijdschr. v. geneesk. 65: 3051-3055, 1921; abst. Ber. ges. Physiol. 8: 433, 1921.
48. Jegorow, Boris: Ein neues Koagulometer, Ztschr. f. d. ges. exper. Med. 29: 543-546, 1922.
49. Feissly, R.: Ein Apparat zur Messung der Blutgerinnungszeit, Schweiz. med. Wchnschr. 52: 300-301, 1922.
50. Aggazotti, A.: La curba di coagulazione del sangue, Boll. Soc. Ital. Biol. sper. 8: 176-179, 1933; abst. Ber. ges. Physiol. 75: 118, 1934.
51. Takasaki, Y.: Über Blutgerinnung nebst Angabe einer neuen Methode zur Bestimmung der Blutgerinnungszeit des Blutes, Mitt. med. Fak. Univ. Tokyo 30: 315-362, 1923; abst. Ber. ges. Physiol. 29: 101, 1925.
52. Szabuniewicz, B.: Eine neue Methode zur Blutgerinnungszeitmessung und zur graphischen Registrierung des Gerinnungsvorganges, Folia haemat. 47: 357-364, 1932.
53. Szabuniewicz, B.: Über die Messung der Blutgerinnungszeit, Arch. f. exper. Path. u. Pharmakol. 187: 497-500, 1937.
54. Riesser, Otto, and Nagel, Alfred: Zur Methodik der Bestimmung der Blutgerinnungs-Geschwindigkeit, Ibid. 179: 743-747, 1935.
55. Cannon, W. B., and Mendenhall, W. L.: Factors Affecting the Coagulation Time of Blood. I. The Graphic Method of Recording Coagulation Used in These Experiments, Am. J. Physiol. 34: 225-231, 1914.
56. Kottmann, K.: Der Koagulovískosimeter mit spezieller Berücksichtigung seiner klinischen Verwendbarkeit für Gerinnungsbestimmungen des Blutes, Ztschr. f. klin. Med. 69: 415-430, 1910.
57. Foster, R. H. K.: A Graphic Method for Measuring the Clotting Time of Blood, J. Lab. & Clin. Med. 16: 505-512, 1931.
58. Takenaka, S.: Studies on Blood Coagulation; a New Coaguloviscosimeter, Acta scholae med. univ. imp. in Kioto 14: 396-400, 1932.
59. Kugelmass, I. N.: Contribution physico-chimique au mécanisme de la coagulation du sang, Arch. internat. de physiol. 21: 139-190, 1923.

60. Schwab, Max: Venenthrombose und Gerinnbarkeit des Blutes, München. med. Wchnschr. 53: 2520-2521, 1906.

61. Kitamura, Nobuharu: Die ultramikroskopisch verschiedenen Formen der Blutgerinnung, Arch. f. d. ges. Physiol. 203: 651-662, 1924.

62. Klinke, K.: Neue Untersuchungen über die zweite Phase der Blutgerinnung, Klin Wchnschr. 10: 869-871, 1931.

63. Elias, G.: Gerinnungsstudien. II. Mitteilung. Eine exacte Methode zur Bestimmung der Blutgerinnung, Ztschr. f. d. ges. exper. Med. 77: 693-705, 1931.

64. Klinke, K.: Gerinnungsstudien. III. Mitteilung. Methodisches zur Tyndallometrie lichtabsorbierender Flüssigkeiten, Ibid. 77: 706-716, 1931.

65. Klinke, K., and Elias, G.: Gerinnungsstudien. IV. Mitteilung. Kinetische Untersuchungen der Fibrinogengerinnung, Ibid. 77: 717-725, 1931.

66. Baldes, E. J., and Nygaard, K. K.: Determination of the Coagulability of the Blood Plasma by the Photo-Electric Cell, Proc. Staff Meet., Mayo Clin. 11: 151-155, 1936.

67. Van Allen, C. M.: Studies in Blood Coagulation. I. Certain Characteristics of Coagulation and Their Measurements, J. Exper. Med. 45: 69-86, 1927.

68. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med. 13: 76-95, 1914.

69. Gram, H. C.: The Coagulation Time of Citrated Plasma on Recalcination, Bull. Johns Hopkins Hosp. 31: 364-368, 1920.

70. Chantemesse: Quoted by Cohen, M. S.[31]

71. Gelera, M.: Un nuovo metodo per saggiare il potere coagulante del sangue. Alcune ricerche speciali eseguite su ammalati, Riforma med. 37: 149-151, 1921; abst. Ber. ges. Physiol. 7: 512-513, 1921.

72. Hardy, W. B.: The Blood Corpuscles of the Crustacea, Together With a Suggestion as to the Origin of the Crustacean Fibrin Ferment, J. Physiol. 13: 165-190, 1892.

73. Meves, Friedrich: Zur Kenntniss der Thrombozytin des Salamanderblutes und ihres Verhaltens bei der Gerinnung, Arch. f. mikr. Anat 68: 311-353, 1906.

74. Pringle, Harold, and Tait, John. Anticoagulants on Frog's Blood, J. Physiol. 40: xxxv-xxxvi, 1910.

75. Pringle, Harold, and Tait, John: Anticoagulants on Frog's Blood. Part II, Ibid. 42: xxxviii-xxxix, 1911.

76. Tait, John: Crustacean Blood Coagulation as Studied in the Arthrostraca, Quart. J. Exper. Physiol. 3: 1-20, 1910.

77. Burke, H. E., and Tait, John: Blood Coagulation as Studied by Intravenous Injection of Tissue Extract, Ibid. 16: 111-127, 1926.

78. Tait, John, and Burke, H. E.: Platelets and Blood Coagulation, Ibid. 16: 129-140, 1926

79. Tait, John, and Green, F.: The Spindle Cells in Relation to Coagulation of Frog's Blood, Ibid. 16: 141-148, 1926.

80. Pickering, J. W.: The Blood Plasma in Health and Disease, New York, 1928, The Macmillan Co, 247 pages.

81. Cramer, W., and Pringle, Harold: On the Coagulation of Blood, Quart J. Exper Physiol. 6: 1-11, 1913.

82. Goddard, C. H.: The Effect of Filtration Through a Berkefeld Filter Upon the Coagulability of Oxalated Plasma, Am. J. Physiol. 35: 333-339, 1914.

83. Bordet, Jules: The Theories of Blood Coagulation, Bull. Johns Hopkins Hosp. 32: 213-218, 1921.

84. Mills, C. A.: The Role of the Platelets in Blood Clotting, Chinese J Physiol. 1: 235-244, 1927.

85. Achard, C., and Aynaud, M.: Action de la gélatine sur les globulins, Compt. rend Soc de biol. 65: 332, 1908.

86. Pickering, J. W.: Modern Conceptions of Blood Clotting, Brit. J. Exper. Biol. 2: 397-411, 1925.

87. Pickering, J. W., and Reeves, H. G.: The Coagulation of Blood Plasma When Divested of Corpuscles, J. Physiol. 60: 276-281, 1925.
88. Pickering, J. W., and Gladstone, R. J.: The Aetiology of Haemophilia, Lancet 1: 602-604, 1925.
89. Jürgens, Rudolf: Verteilung der Eiweisskörper im Blute bei hämorrhagischen Diathesen, Deutsches Arch. f. klin. Med. 171: 378-390, 1931.
90. Howell, W. H.: The Nature and Action of the Thromboplastic (Zymoplastic) Substance of the Tissues, Am. J. Physiol. 31: 1-21, 1912.
91. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extracts), Ibid. 114: 282-296, 1936.
92. Ferguson, J. H.: An Experimental Analysis of Coagulant Activation, Ibid. 117: 587-595, 1936.
93. Bizzorero, Julius: Über einen neuen Formbestandtheil des Blutes und dessen Rolle bei der Thrombose und der Blutgerinnung, Virchows Arch. f. path. Anat. 90: 261-332, 1882.
94. Fåhraeus, Robin: Über die Ursachen der verminderten Suspensionsstabilität der Blutkörperchen während der Schwangerschaft, Biochem. Ztschr. 89: 355-364, 1918.
95. Fåhraeus, Robin: The Suspension Stability of the Blood, Acta med. Scandinav. 55: 3-228, 1921.
96. Fåhraeus, Robin: The Suspension Stability of the Blood, Physiol Rev. 9: 241-274, 1929.
97. Horwitz, S.: Neuere Untersuchungen an Blutplättchen. II. Mitteilung. Das Thrombocytenvolumen, Ztschr. f. d. ges. exper. Med. 73: 432-451, 1930.
98. Gabbe, Erich: Untersuchungen über die elektrische Ladung der Blutkörperchen. I. Mitteilung, Ibid. 33: 324-338, 1923.
99. Gabbe, Erich. Untersuchungen über die elektrische Ladung der Blutkörperchen. II. Mitteilung, Ibid. 33: 276-296, 1924
100. Schulte, Hans: Beitrag zu den physikalisch-chemischen Grundlagen der Thrombose, Med. Klin. 22. 1003-1004, 1926.
101. Starlinger, W., and Sametnik, S.: Über die Entstehungsbedingungen der spontanen Venenthrombose, Klin. Wchnschr. 6: 1269-1272, 1927
102. Starlinger, Wilhelm: Über die klinische Bedeutung des physikochemischen Zustandes des zirkulierenden Eiweisskörper des Blutes und Gewebes, Zentralbl. f. inn Med. 48: 418-431, 1927.
103. Stern, N. S.: Factors Affecting the Coagulation Time of Blood VIII. The Influence of Certain Metals and the Electric Current, Am. J Physiol. 40: 186-193, 1916
104. Takashima, M.: Die Beziehungen der Körperfremden Oberfläche zur Blutgerinnung, Acta scholae med. univ. imp. in Kioto 16. 257-261, 1934.
105. Leiri, F.: Sur la coagulation du sang, Acta med. Scandinav. 82. 268-275, 1934.
106. Michaelis: Quoted by Starlinger, Wilhelm [102]
107. Green, J. R.: On Certain Points Connected With the Coagulation of the Blood, J. Physiol. 8: 354-371, 1887.
108. Arthus, Maurice, and Pagès, Calixte Nouvelle théorie chimique de la coagulation du sang, Arch de physiol. norm et path 2: 739-746, 1890
109. Arthus, Maurice: La coagulation du sang et les sels de chaux (réfutation expérimentale des objections d'Alexander Schmidt), Ibid. 8: 47-61, 1896.
110 Pekelharing, C. A.: Untersuchungen über das Fibrinferment (1892), quoted by Arthus, Maurice [109]
111 Vines, H. W. C: The Coagulation of the Blood. Part I The Role of Calcium, J. Physiol. 55: 86-99, 1921.
112. Collingwood, B J: Some Considerations Concerning the Calcium Content of the Blood and the Influence of Small Variations in Its Amounts on the Coagulation Time, J. Physiol. 38: lxix-lxx, 1909.
113. Collingwood, B. J.: Blood Coagulation and Calcium Ions, Ibid 38: lxxix, 1909.

Stuber, Bernhard, and Sano, Minoru: Untersuchungen zur Lehre von der Blutgerinnung. VII. Mitteilung. Über die Rolle des Kalks bei der Blutgerinnung, Biochem. Ztschr. 134: 260-268, 1922-1923.

Stuber, Bernhard, and Focke, Fritz: Untersuchungen zur Lehre von der Blutgerinnung. XI. Mitteilung. Über die Entbehrlichkeit des Kalkes für dem Gerinnungsprozess, Ibid. 154: 77-81, 1924.

Nordbo, Ragnvald: Über die Aktivitatskoeffizienten der Kalziumionen und der Oxalationen im Plasma und uber die Bedeutung der Kalziumionenkonzentration fur die Gerinnung des Blutes, Berlin and Leipzig, 1936, Walter de Gruyter & Co., 46 pages

Ransmeier, J. C., and McLean, F. C.: The Relationship of Calcium Ion Concentration to the Coagulation of Citrated Plasma, Am. J. Physiol. 121: 488-494, 1938.

Lebel, H., Schonheyder, F., and Muus, J.: The Relationship Between Coagulation Time of Plasma and the Concentration of Ionized and Unionized Calcium, Skandinav. Arch. f. Physiol. 78: 179-188, 1938.

Mulli, K., Pillewizer, T., and Laves, W.: Über die Reaktionen einiger blutgerinnungshemmender Stoffe mit Calcium-Ion in wasseriger Losung, Arch. f. exper. Path. u. Pharmakol. 172. 384-388, 1933.

Addis, T.: The Pathogenesis of Hereditary Haemophilia, J. Path. & Bact. 15: 427-452, 1911.

Howell, W. H., and Cekada, E B.. The Cause of the Delayed Clotting of Hemophilic Blood, Am. J. Physiol. 78: 500 511, 1926.

Smith, C. H : Studies in Calcium and Blood Coagulation (With Special Reference to the Use of Salted Plasma as a Method of Estimating Clotting Time), Am. J. M. Sc. 169: 572 583, 1925.

Fischer, Albert. Ein neuer Blutgerinnungsapparat, Arch f. d. ges. Physiol. 225: 737-739, 1930.

Bancroft, F. W., Kugelmass, I N., and Stanley-Brown, Margaret: Evaluation of Blood Clotting Factors in Surgical Diseases; With Special Reference to Thrombosis and Embolism and Certain Bleeding Conditions, Ann. Surg. 90. 161-189, 1929.

Lewisohn, Richard: Haematologic Studies as a Basis for Determining the Risk of Post-Operative Haemorrhage in Jaundice Patients, Ibid 94. 80 86, 1931.

Nygaard, K. K.: Coagulability of Blood Plasma, Proc. Staff Meet, Mayo Clin. 7: 544 547, 1932

Nygaard, K. K.: Coagulability of Blood Plasma a Method of Determining Hemorrhagic Tendency of Jaundiced Patients, Ibid. 7: 691 696, 1932.

Nygaard, K. K Coagulability of Blood Plasma; Remarks on the Technic of Its Determination, Ibid. 9 151-156, 1934.

Nygaard, K. K . Coagulability of the Blood Plasma and Changes in the Number of Platelets in Thrombocytopenic Purpura, Ibid. 9: 492-496, 1934.

Bancroft, F. W., Stanley-Brown, Margaret, and Quick, A. J.· Postoperative Thrombosis and Embolism, Am J. Surg 28· 648 668, 1935.

Dyckerhoff, H , v. Behm, W., Goossens, N., and Miehler, H. Über die Gerinnung des Blutes. II, Biochem. Ztschr 288: 271 291, 1936

Kurten, H. F., and Harzer, R. Mitteilung uber die Konstanz des Blutgerinnungssystems, gepruft an Oxalatplasmen Gesunder und Kranker, Ztschr. f d. ges exper. Med 102. 449-460, 1938.

Mayer, André. La coagulation du plasma sanguin Etude ultramicroscopique, Compt. rend Soc de biol. 63: 658 660, 1907

Cesana, G.: Quoted by Stubel, Hans [135]

Stubel, Hans. Ultramikroskopische Studien über Blutgerinnung und Thrombocyten, Arch. f. d. ges Physiol. 156 361 400, 1914.

Stübel, Hans: Die Fibringerinnung als Krystallisationsvorgang, Ibid. 181· 285-309, 1920

137. Howell, W. H.: The Clotting of Blood as Seen With the Ultramicroscope, Am. J. Physiol. 35: 143-149, 1914.
138. Kitamura, Nobuharu: Die ultramikroskopisch verschiedenen Formen der Blutgerinnung, Arch. f. d. ges. Physiol. 203: 651 662, 1924.
139. Wolvius, B. J.: Quoted by Festen, H.[141]
140. Baldes, E. J., and Nygaard, K. K.: Determination of the Coagulability of the Blood Plasma by the Photo Electric Cell, Proc. Staff Meet., Mayo Clin. 11: 151-155, 1936.
141. Festen, H.: Eine objektive Methode zur Bestimmung der Blutgerinnungszeit und Gerinnungsdauer, München. med. Wchnschr. 2: 1370 1371, 1937.

CHAPTER II

GENERAL CONSIDERATIONS OF THE PHOTO-ELECTRIC PRINCIPLE

The discovery of the so-called photo-electric effect is credited to Heinrich Herz,[1] who in 1887 performed his classical experiments, opening up an entirely new field of physical investigation. The further initial studies were carried out by Hallwachs,[2] who in 1888 discovered the so-called Hallwachs' effect which led directly to the development of the alkali photo-electric cells. These cells could not serve any definite practical purpose at the time. In 1889 Elster and Geitel[3] constructed the first photo-electric device that permitted operation over periods sufficiently long for measurements.

Since then the photo-electric effect has been the subject of theoretic and practical investigations by numerous physicists all over the world. The last two decades particularly have witnessed rapid progress in the perfection of the various types of photo-electric cells, eliminating many of the sources of unreliability and disadvantages and raising the sensitivity and practical applicability of the photo-electric cells to include responses to radiant energy in the visible as well as invisible part of the spectrum.

The present chapter is concerned chiefly with densitometric and colorimetric measurements of medical consequence. The subsequent considerations are presented with this point in mind, omitting problems that are outside the field of medical photometry.

The incapability of the human eye to match colors and determine light intensities to any accurate degree is indisputable. For two main reasons the eye is not a reliable judge in this respect:

First, the response of the eye is not proportionate to the intensity of the light. For a given intensity the increment required for detection by the eye is proportionate to the intensity itself (Zworykin and Wilson[4]) This means that the eye becomes less sensitive to further increase of light intensity the brighter the source becomes It is understood that quantitative determinations based on colorimetric reactions consequently are subject to no small number of errors when undertaken by visual observations. This difficulty is overcome to a great extent by the use of the modern photo-electric cells through their linear dependence of the response of the cells to the intensity of the illumination. The latter physical property of the cells is not to be accepted, however, without reservation The photo-electric response depends largely on the internal and external resistance of the cell However, within the range of illumination generally used in colorimetric and densitometric investigations employing the blocking layer type of cells, direct proportionality between illumination and response of the cell is obtainable.

Second, the eye is unequally sensitive toward colors. The practical consequence of this will be, for example, that, if a red light and a yellow light of the same intensity are compared by visual observation, the yellow light will appear brighter. Radiation of energy must therefore be differentiated from the luminous radiation as determined visually. Actual comparison of the luminous intensities of the two sources consequently can be made only when the sources are the same color or reduced to the same color by means of suitable compensating filters. The spectral sensitivity of the photo cells will be of decisive significance since the agreement with visual measurements occurs only when the measuring device has the same or similar spectral sensitivity as the human eye. Photo-electric measurements, however, are far superior since the individual observer seldom knows the deviation of his own eyes from the mean eye sensitivity and since visual heterochromatic photometry fatigues the eye greatly.

For years investigative minds have concentrated on the possibility of obtaining a perfect technical substitute for the human eye as a measuring instrument. The photo-electric cell at present can be said to represent only partly such an ideal substitute. It can be employed as such with great advantage, however, when accounting for some of its shortcomings.

The principal modus operandi of the photo-electric cell is the production of an electric current by exposure of its photosensitive pole to light, thus demonstrating the reversible nature of the relation between an electric current and light The voltage or the amperage of the current may be measured by suitable registering instruments inserted in the current circuit with or without amplification of the current.

Any medium present or introduced between the source of light and the photosensitive pole of the cell will modify the transmitted light; that is, result in a change in the electric current as measured by the registering instrument. This relative change in the electric current forms the basis of the quantitative photo-electric measurement In order to perform satisfactory observations it is necessary to take into account essential physical characteristics of the photo-electric cell which mainly are as follows (1) the spectral sensitivity varying with different types of cells; (2) irreversible change of sensitivity over longer periods of time; (3) rapid, reversible variations in current output (the element of fatigue and recovery); (4) variation in the internal resistance of the cell resulting from incorrectly selected degree of illumination; and (5) the influence of temperature variations on the current output of the cell Other possibilities of errors not directly related to the photo-electric cell must also be taken into account. These are· (1) inconstancy of the source of light; (2) lacking or deficient optical arrangement; (3) instability of the technical apparatus (mechanical disturbances through shaking), and (4) nonhomogenous or nonparallel plane sides of test tubes, cuvettes, or absorption cells as used in photometry. These sources of errors have been considered in detail in a previous communication [116]

As to the applicability of the photo-electric cell, we find that it has been employed in a great number of investigative problems related to biology,

physiology, pathology, bacteriology, and other fields more or less directly concerned with problems of medical significance.

Noyons[5] appeared to have been the first to introduce this new technique to medical research. In 1921 he attempted an estimation of the amount of hemoglobin liberated in suspensions of erythrocytes by means of a selenium photo-electric cell, but he had to abandon further investigations because of the unreliability of the cells at that time. In 1924 Mellanby assembled a photo-electric apparatus which was used by Ponder[6, 7] in some of his important studies on the kinetics of the phenomenon of hemolysis. In 1926 Reimann[8] applied photo-electric colorimetry to sugar determinations. Not until 1929, however, was a standardized and reliable photo-electric colorimeter constructed, the later photelometer of Sheard and Sanford.[9] Since then a great number of similar instruments for different purposes have been introduced. They are either of the so-called one-cell or two-cell principle.

The one-cell photo-electric instrument consists essentially of one photo-electric cell, a source of light of constancy, and an absorption cell containing the specimen to be tested By comparing the current output at known standards with that at unknown concentrations a quantitation can be reached. This type of apparatus is probably the one most commonly in use Instruments of the one-cell type are those of Sheard and Sanford,[9] Ellis,[11] Holiday and coworkers,[12] Evelyn,[13] and many others.

The two-cell instruments consist essentially of two photo-electric cells, a source of light simultaneously illuminating both cells, a standard absorption cell, and an arrangement by which it is made possible readily to estimate the difference in transmitted light reaching the two cells Instruments of this type have been constructed by Exton,[14] Lange,[15] Goudsmit and Summerson,[16] Northup, Hawk, and Andes,[17] and others. As to the choice of instruments, either type has certain advantages, the two-cell type probably being preferable to workers less experienced in photo-electric investigations A knowledge of the various sources of errors concerned in the one-cell type of apparatus permits its use with a great percentage of accuracy when suitable measures are taken to counteract the sources of errors.

Numerous substances have been subjected to photo-electric, densitometric, and colorimetric investigations In colorimetry quantitation of substances possessing characteristic absorption bands has been particularly popular, the results essentially depending upon the proper selection of filters Mention may be made of investigations concerning blood sugar, blood iron, hemoglobin, hematoporphyrins, creatinine, creatine, guanidine, calcium, bilirubin, estrin, vitamins A and B (lactoflavin, aneurin), ascorbic acid, glucose and alcohol in urine, carotene, sulfanilamide, sulfapyridine, and many others

One of the common characteristics of the quantitative estimations mentioned is that the specimen under investigation exhibits only one final characteristic of transillumination. This simplifies greatly the quantitative estimation. The latter follows from one final reading of the light transmitted through a system in colorimetric equilibrium As an example may be mentioned the

photo-electric estimation of hemoglobin after complete hemolysis of the erythrocytes through the action of a hemolyzing agent. This and similar investigations may be termed *stationary photo-electric determinations.*

Retaining the example chosen, it will be readily understood that by starting one's photo-electric observations before the completion of the hemolysis one would note a change in the transmitted light from one moment to the next until complete hemolysis had been obtained. This procedure would be of no value if one's interest is centered on the quantity of hemoglobin present in the specimen. Quite different, however, is the condition if one is interested primarily in the rate at which hemolysis occurs. If photo-electric readings are taken at frequent intervals from the beginning of the reaction, the plotting of the readings will describe a geometric curve which will represent a function of the quantity of hemolyzed material in relation to the time factor; in short, *represent a velocity curve of the progressing reaction. This and similar photo-*electric investigations may be termed *progressing photo-electric determinations.* A typical reaction of this nature is represented by the coagulability of the blood, the reaction to be the subject of study in the present work. The great practical advantages of photo-electric investigative technique are readily demonstrated by these progressive processes. The success of these researches will naturally depend to a large extent on a successful technique of registering or recording the progressive changes. In relatively slowly progressing processes satisfactory results may be obtainable by visual readings of the registering instrument. In faster reactions this is possible only when utilizing automatic recordings of the process by photokymographic or similar arrangements. The photelgraph, to be described later, includes provisions for automatic recording.

In surveying the various photo-electric methods as utilized in investigations of medical importance, it may be natural to divide them into these two different types of reactions, the stationary and the progressing. A survey of the various methods as published up to the beginning of 1940 is presented in Tables II and III

TABLE II

STATIONARY PHOTO ELECTRIC METHODS

		(ric scopometer)
1933	Sanford, Sheard, and Osterberg[21]	Hemoglobin, blood sugar, creatinine *(photelometer)*
1933	Colla[22, 23]	Chlorophyll
1933	Alper and Sterne[24]	Bacterial population
1933	Naumann[25]	Iron, manganese, phenol, aluminum
1933	Lesure, Thomas, and Levagne[26]	Calcium, oxalic acid, sulfur, flocculation reactions
1933	Bendig and Hirschmuller[27]	Iron, manganese
1933–1936	Willer and Heinemann[28]	Influence of suspended matter in water on lagoon sickness

TABLE II—CONT'D

YEAR	INVESTIGATORS	SUBJECT OF INVESTIGATION
1934	Seraux[29]	Arsenic
1934	Awtonomowa and Stessel[30]	Bacterial populations
1934	Roeder[31]	Proteins in spinal fluids
1934	Stier, Arnold, and Stannard[32]	Bacterial populations
1934	Griebel[33]	Chlorogenic acid
1934	Cerny[34]	Silicic acid
1934	Obermer and Milton[35]	Urea
1934	Fegler and Modzelewski[36]	Oxygen and carbon dioxide in air
1934	Bruckner and Becker[37]	Sugar
1934	Ellis[11]	Turbidity measurements
1935	Armstrong and Kuder[38]	Nonprotein nitrogen, hemoglobin, creatinine, urea, nitrogen, blood sugar, uric acid, phenolsulfonphthalein test
1935	Rothschild[39]	Phosphoric acid
1935	Zinzadse[40]	Phosphorus, arsenic
1934, 1935	Yamamoto and Abe[41]	Methylene blue, chinone yellow, eosine red
1934, 1935	Uzel[42]	Mercury
1935	Kramer[43]	Nitrates
1935	Holiday, Kerridge, and Smith[12]	Hemoglobin
1935	Goudsmit and Summerson[16]	Creatinine
1935	Cohen[44]	Vitamin B, lactoflavin, fluorescence determinations
1936	Jansen[45]	Vitamin B, aneurin, fluorescence determinations
1936	Longsworth[46]	Bacterial populations
1936	Eisler, Rosedahl and Theorell[47]	Copper (hematopoiesis)
1936	Diller[48]	Uric acid, sugar, creatinine
1936	Evelyn[13]	General colorimeter
1937	Evelyn and Cipriani[49]	Microcolorimeter
1937	Malloy and Evelyn[50]	Bilirubin
1937	Venning, Evelyn, Harkness, and Browne[51]	Estrin in urine
1938	Evelyn and Gibson[52]	Improved colorimeter
1937	Ragno[53]	Glucose in urine
1937	Pilcher and Sheard[54]	Blood loss during operations
1937	Lebowich, Lebowich, and Dinburg[55]	Dextrose, creatinine, uric acid
1937	Stevens and Turner[56]	Hematoporphyrine
1937	Dobriner, Strain, and Localio[57]	Coproporphyrine
1937	Pollès and Frocrain[58]	Glucose, chlorine in blood
1937	Hoffman[59, 60]	Glucose in blood and urine, potassium in serum
1937	Stueck, Flaum, and Ralli[61]	Carotene in serum
1938	Malloy and Evelyn[62]	Bilirubin in bile and meconium
1938	Dann and Evelyn[63]	Vitamin A
1938	Gibson and Evelyn[64]	Blood volume
1938	Kennedy and Millikan[65]	Blood volume
1938	Hare and Phipps[66]	Creatinine
1938	Uvidich[67]	Creatinine
1938	Mindlin and Butler[68]	Ascorbic acid in blood plasma
1938	Gibson and Blotner[69]	Ethyl alcohol in urine
1938	Bessey[70]	Ascorbic acid in turbid solutions
1938	Evelyn and Malloy[71]	Oxysulph-hemoglobin
1939	Havemann[72, 73]	Methemoglobin
1939	Rosenfeld[74]	Sulfanilamide, sulfapyridine, iron, copper, creatinine
1938	Andes and Northup[77]	Hemoglobin, blood, iron
1939	Northup, Hawk, and Andes[76]	Nonprotein nitrogen, urea, creatine, creatinine, guanidine, amino acids, nitrogen in blood
1939	Andes and Northup[75]	Blood glucose, cholesterol, serum phosphorus, plasma proteins, and urine sugar
1940	Medes and Stavers[78]	Sulfates in biologic fluids *(photelometer)*
1940	Johnson, Aude, and Sorum[79]	Readings of the Lange test *(photelometer)*

TABLE III

PHOTO ELECTRIC METHODS CONCERNING PROGRESSING PROCESSES

YEAR	INVESTIGATORS	SUBJECT OF INVESTIGATION
1924–1932	Ponder[7]	Phenomenon of hemolysis
1928	Kesten and Zucker[80]	Phenomenon of hemolysis
1931	Harvey and Snell[81, 82]	Bioluminescence studies
1932	Collatz and Weber[83]	Studies on venous pulse
1932	Netter and Ørskov[84]	Phenomenon of hemolysis; contraction of nerve-muscle preparations
1932	Nicolai[85-88]	Reversible oxidoreduction of hemoglobin in vivo
1934	Bonsmann[89]	Blood pressure in rats
1934–1935	Kramer[90, 91]	[illegible]
1934–1935	Matthes[92, 93]	[illegible]
1934–1936	Ørskov[94-96]	[illegible]
1935	Baumann[97]	[illegible]
1935	Marrazzi[98]	Movements of the gall bladder
1935	von Muralt[99]	Metabolism of muscles in situ
1935	Gradstein[100]	Measurement of the comb of capons in the assay of male sex hormones
1936	Barach and Eckman[101]	[illegible]
1936	Swedin[102]	[illegible]
1936	Baldes and Nygaard[103, 107]	[illegible]
1936–1938	Hartmann and Wullstein[104]	[illegible]
1937	Festen[106]	[illegible]
1937–1938	Hertzman[105, 108-112]	[illegible]
1938	Gross[113]	[illegible]
1938	Nygaard and Guthe[114]	[illegible]
1938	Evelyn, Malloy, and Rosen[115]	[illegible] ascorbic acid
1939	Nygaard[116]	Apparatus applicable to progressing processes (*photelgraph*)
1938–1939	Guthe and Nygaard[117-119]	Photochemical, quantitative oxidoreduction of ascorbic acid
1939	Nygaard[120]	Quantitative determination of prothrombin
1940	Nygaard, Gathe, and Guthe[121]	Quantitative determination of blood fibrin

It is to be expected that the photo-electric technique will be extended in the future to a great variety of many more progressive reactions. These processes, as investigated by the photo-electric technique, may raise rather complex problems inasmuch as the investigative apparatus may or may not constitute an active part of the process through the indispensable source of light of the technical device It may serve a definite purpose at this point to try a tentative classification of the progressive reactions

1. Processes may result from the interaction of chemical reactions resulting in the formation of substances with extinction coefficients different from those of the original components Examples Formation of fibrin by recalcification of blood plasma; the permanent oxidation-reduction of hemoglobin-oxyhemoglobin in vivo.

2. Progressive changes in biologic systems caused by biologic or chemical factors Examples. Volumetric changes of erythrocytes, aggregation formation of erythrocytes; hemolysis by lytic chemicals, hemolysis in complement fixation reactions (Wassermann reaction), flocculation, and precipitation tests; growth of bacterial population, and others.

3. Gross volume changes of objects under investigation Examples: Movements of the vocal cords; contraction of the gall bladder; plethysmographic studies; movements of muscles in physiologic experiments and others.

4. Progressive reactions induced or influenced by radiant energy acting upon chemical or biologic systems. Examples: Hemolysis by ultraviolet rays; photoreduction of aneurin; photo-oxidation of ascorbic acid.

This brief summary of the various photo-electric methods in use in fields closely related to medical problems may give an impression of the great and varied applicability of the photo-electric technique and its potentialities for further research.

References

1. Herz, Heinrich: Quoted by Zworykin, V. K, and Wilson, E. D.[4]
2. Hallwachs, Wilhelm: Quoted by Zworykin, V. K., and Wilson, E. D.[4]
3. Elster, Julius, and Geitel, Hans. Quoted by Zworykin, V. K, and Wilson, E. D.[4]
4. Zworykin, V. K., and Wilson, E. D.: Photocells and Their Application, ed. 2, New York, 1934, John Wiley and Sons, Inc., 348 pages.
5. Noyons, A. K.: L'étude de l'hémolyse par l'extinctiométrie, Arch. internat. de physiol. 18: 250-268, 1921.
6. Ponder, E. H.: The Erythrocyte and the Action of Simple Haemolysins, Edinburgh & London, 1924, Oliver & Boyd, 192 pages.
7. Ponder, E. The Kinetics of Hemolysis, Physiol. Rev. 16: 19-51, 1932.
8. Reimann, S. P.: The Photoelectric Cell as a Colorimeter, Proc. Soc. Exper. Biol. & Med. 23: 520-523, 1926.
9. Sheard, Charles, and Sanford, A H. A Photo-Electric Hemoglobinometer; Clinical Applications of the Principles of Photo Electric Photometry to the Measurement of Hemoglobin, J. Lab. & Clin. Med. 14. 558-574, 1929.
10. Sanford, A. H., and Sheard, Charles The Determination of Hemoglobin With the Photo-Electrometer, Ibid. 15: 483 489, 1930.
11. Ellis, M. M: A Photoelectric Apparatus for Turbidity and Light Penetration Measurement, Science 80: 37, 1934.
12. Holiday, E. R., Kerridge, Phyllis M. T, and Smith, F. C.: Amount of Haemoglobin in the Blood, Lancet 2: 661-665, 1935.
13. Evelyn, K. A. A Stabilized Photoelectric Colorimeter With Light Filters, J. Biol. Chem. 115. 63 75, 1936.
14. Exton, W. G.: The Photoelectric Scopometer, Am. J. Clin. Path. 2: 411-420, 1932.
15. Lange, Bruno: Photoelements and Their Application, New York, 1938, Reinhold Publication Corporation, 297 pages.
16. Goudsmit, Arnoldus, and Summerson, W. H. A Variable Layer Photoelectric Comparison Photometer; a New Type of Photoelectric Colorimeter, J. Biol. Chem. 111: 421-433, 1935.
17. Northup, D., Hawk, F. L, and Andes, J E. Photoelectric Colorimetry II. Quantitative Determination of Blood Nonprotein Nitrogen, Urea, Creatinine, Creatine, Guanidine and Amino Acids, J Lab. & Clin. Med. 24. 432-438, 1939
18. Andrejew, N. N. Eine neue Methode zur Untersuchung der kolloiden Systeme, Kolloid Ztschr. 43 14-17, 1927
19. Fiske, C, and Subbarow, J.. Quoted by Lange, Bruno[15]
20. Exton, W. G., and Rose, A R.: The Clinical Partition of Blood Protein by Scopometry. II. Method, J. Biol. Chem. 97 xxvii-xxix, 1932.
21. Sanford, A. H., Sheard, Charles, and Osterberg, A. E · The Photelometer and Its Use in the Clinical Laboratory, Am. J Clin. Path 3: 405-420, 1933.

22. Colla, Silvia: Elettrofotometrie con luci monocromatica inmiscugli de piu sostanze. 1) L'impiez della cella fotoelectrica per dosaggi con luci selezionati, Boll. Soc. ital. biol. sper. 8: 315 317, 1933.
23. Colla, Silvia: 2) Totolozione de miscele di clorofilla in presenza di xantofillia e carotina, Ibid. 8: 317 321, 1933.
24. Alper, T., and Sterne, M.: The Measurement of the Opacity of Bacterial Cultures With a Photoelectric Cell, J. Hyg. 33: 497 509, 1933.
25. Naumann, E.: Quoted by Lange, Bruno [15]
26. Lesure, A., Thomas, A., and Levagne, J.: Les cellules photoélectriques et leur applications en chimique biologique, J. de pharm. et chim. 17: 5 21; 49 61, 1933.
27. Bendig, M., and Hirschmuller, H.: Quoted by Lange, Bruno.[16]
28. Willer, A., and Heinemann, E.: Quoted by Lange, Bruno [15]
29. Sevaux, A.: Emploi du comparateur photo électrique pour le dosage de petites quantités d'arsenic, Compt. rend. Soc. de biol. 115: 377-380, 1934.
30. Awtonomowa, E. S., and Stessel, T. A.: Nephelometrische Methode zur Bestimmung der Anzahl der Mikrobenleiber in bakteriellen Suspensionen mit Hilfe einer Photozelle, Biochem. Ztschr. 274: 220 230, 1934.
31. Roeder, F.: Ueber die experimentellen Grundlagen einer lichtelektrischen Liquor eiweissbestimmung mit Hilfe von Sperrschiechtphotozellen, Ztschr. f. d. ges Neurol. u. Psychiat. 151: 718 728, 1934.
32. Stier, T. J. B, Arnold, W., and Stannard, J. N.: A Photoelectric Densitometer for Use With Suspensions, J. Gen. Physiol. 17. 383-392, 1934.
33. Griebel, C.: Quoted by Lange, Bruno.[15]
34. Cerny, M.: Quoted by Lange, Bruno.[15]
35. Obermer, E., and Milton, R.: Un densitometro fotoelettrico con una nuova technica per il dosaggio dell'urea nell'urina, Diag e tec. di lab. 5: 741-752, 1934.
36. Fegler, J., and Modzelewski, T.: Méthode photoélectrique de détermination de la teneur de l'air en O_2 et CO_2, Compt. rend. Soc. de biol. 116: 244-248, 1934.
37. Bruckner, A., and Becker, S. Quoted by Lange, Bruno [15]
38. Armstrong, E. L., and Kuder, M. L.: The Limitations of Colorimetric Analysis by Present Methods, J. Lab & Clin. Med. 21: 181-187, 1935
39 Rothschild, F.. Quoted by Lange, Bruno [15]
40. Zinzadse, Ch.. Quoted by Lange, Bruno.[15]
41 Yamamoto, K., and Abe, M.: Quoted by Lange, Bruno [15]
42. Uzel, R: Quoted by Lange, Bruno.[15]
43. Kramer, K.: Quoted by Lange, Bruno [15]
44 Cohen, F. H.: Simple Apparatus Employing a Selenium Cell for Objective Fluorescence Measurements, Chem. Abstr. 29: 3605, 1935.
45 Jansen, B C P.: A Chemical Determination of Aneurin (Vitamin B_1) by the Thiocrome Reaction, Rec d trav chim d. Pays Bas. 55. 1046 1052, 1936
46. Longsworth, L. G.: The Estimation of Bacterial Populations With the Aid of a Photoelectric Densitometer, J. Bact. 32: 307-328, 1936.
47. Eisler, B., Rosedahl, K. G, and Theorell, H Über die Mikrobestimmung des Kupfers mit Hilfe der lichtelektrischen Photometrie, Biochem Ztschr 285: 76-77, 1936.
48. Diller, I M.: Photoelectric Colorimeter, J. Biol. Chem 115: 315-322, 1936.
49. Evelyn, K. A., and Cipriani, A. J.: A Photoelectric Microcolorimeter, J. Biol. Chem. 117: 365 369, 1937.
50. Malloy, H. T., and Evelyn, K. A: The Determination of Bilirubin With the Photoelectric Colorimeter, Ibid 119: 481 490, 1937
51. Venning, E H., Evelyn, K A., Harkness, E. V, and Browne, J S. L.: The Determination of Estrin in Urine With the Photoelectric Colorimeter, Ibid. 120. 225-237, 1937.
52 Evelyn, K. A., and Gibson, J. G.: A New Type of Absorption Cell for the Photoelectric Microcolorimeter, Ibid. 122. 391-394, 1938.

102. Swedin, B.: Untersuchungen ueber den Aggregationsmechanismus der Erythrocyten, Stockholm, 1936, Centraltryckeriet, 91 pages.
103. Baldes, E. J., and Nygaard, K. K.: Determination of the Coagulability of the Blood Plasma by the Photo-Electric Cell, Proc. Staff Meet., Mayo Clin. 11: 151-155, 1936.
104. Hartmann, W., and Wullstein, H.: Motor Process of Vibrating Labia Vocalia of Laryngeal Preparations Examined by Improved Photoelectric Cell, Arch. f. Ohren-, Nasen- u. Kehlkopfh. 144: 348-360, 1938.
105. Hertzman, A. B.: Photoelectric Plethysmography and Sphygmography in Man and Animal, Am. J. Physiol. 123: 99-100, 1938.
106. Festen, H.: Eine objektive Methode zur Bestimmung der Blutgerinnungszeit und Gerinnungsdauer, Munchen. med. Wchnschr. 2: 1370-1371, 1937.
107. Nygaard, K. K., and Baldes, E. J.: Interpretation and Clinical Significance of Coagel grams in Obstructive Jaundice, Proc. Staff Meet., Mayo Clin. 11: 705-709, 1936.
108. Hertzman, A. B.: Photoelectric Plethysmography of the Nasal Septum in Man, Proc. Soc. Exper. Biol. & Med. 37: 290-292, 1937.
109. Hertzman, A. B.: Photoelectric Plethysmography of Fingers and Toes in Man, Ibid. 37: 529-534, 1937.
110 Hertzman, A. B.: Comparative Estimation of Blood Supply of Skin Areas From Photoelectrically Recorded Volume of Pulse, Proc. Soc Exper Biol. & Med. 38: 562-564, 1938.
111. Hertzman, A. B. Skin Vascular Reactions to Cold Pressor Test, Ibid 38: 628 629, 1938
112. Hertzman, A. B.: The Blood Supply of Various Skin Areas as Estimated by the Photo electric Plethysmograph, Am J. Physiol 124: 328-340, 1938
113 Gross, Franz: Über die Reduktionszeit des Blutes, Ztschr f. d. ges. exper. Med. 102: 766-777, 1938.
114. Nygaard, K. K., and Guthe, Th.: Automatic Recording of the Wassermann Reaction by a Photoelectric Principle (Photelgraph), Acta med Scandinav. 97: 293 306, 1938
115. Evelyn, K. A., Malloy, H. T, and Rosen, Charles: The Determination of Ascorbic Acid in Urine With the Photoelectric Colorimeter, J. Biol. Chem 126: 645 654, 1938
116. Nygaard, K. K: A New Apparatus for Photoelectric Recording of the Coagulability of the Blood and Other Progressing Processes, J Lab. & Clin Med. 24: 517-522, 1939
117 Guthe, Th, and Nygaard, K K. The Photoelectric Determination (Photelgraph) of Ascorbic Acid, Chem. & Indust. 57: 1195-1199, 1938
118. Guthe, Th, and Nygaard, K K. Studies on Ascorbic Acid by Means of the Photelgraph I General Consideration on the Photoelectric Principle and Its Applicability to the Study of Progressive Processes, Acta. med. Scandinav. 99: 1 11, 1939.
119. Guthe, Th., and Nygaard, K K.: II. Quantitative Applicability and Photochemistry of Reversible Oxidation-Reduction Systems of Ascorbic Acid-Methylene Blue, Ibid. 101 40-61, 1939
120. Nygaard, K. K.: Studies on the Velocity of Blood Coagulation by the Photoelectric Principle, With Particular Reference to Prothrombin Determinations, Lancet, in press
121. Nygaard, K. K, Gathe, R, and Guthe, Th Quantitative Determination of Blood Fibrin by the Photoelectric Method, Scandinav. Arch f Physiol, in press

CHAPTER III

THE PHOTELGRAPH

After having worked out a standardized technique for the determination of the coagulability of the recalcified plasma and indicated its applicability to certain clinical conditions, it was felt desirable to improve upon the accuracy of the test by substituting the regular visual reading of the coagulation process with a more satisfactory reading technique. The observation that an increased density occurs during the coagulation of recalcified plasma suggested the possibility of registering this change by the photo-electric technique. The first apparatus designed for this purpose was very simple. Its essential parts are indicated in Fig. 5. It will be understood that the progression of the coagulation, that is, the increasing density of the specimen, would result in a gradual change in the movement of the amperemeter needle. In order to obtain a picture of the progression, a reading of the amperemeter needle was undertaken every 10 seconds and the readings were plotted on diagrammatic paper. The plotted curves indicated the velocity of the progressive process. It is plain that the reading of the movements of the amperemeter needle is subject to the errors of the personal investigator, the very point which our technical device sought to exclude. For this reason an apparatus was assembled which permitted an automatic graphic recording of the progressive reaction. This apparatus was termed coagelgraph, to differentiate it from the original device, the coagelometer. The essential principles of the coagelgraph are included in the apparatus shortly to be described. This work was done originally at The Mayo Clinic in cooperation with E. J Baldes[1] and the Department of Physics and Biophysical Research.

The original arrangement of the coagelgraph required a darkroom in which to perform the investigations Because of the sensitivity of the galvanometer used for these studies, a very stable support for it was essential, requiring a cement floor in a room in the basement of the hospital. If the procedure was to have a more practical use, it would be necessary to design an apparatus which satisfied certain practical requirements The following points were considered of basic importance. Arrangements of the various parts of the apparatus into one transportable unit, requiring a minimum of space, to be operated in any laboratory without the necessity of a darkroom, the equipment still retaining the accuracy and stability of the original arrangement Efforts to overcome these technical difficulties resulted in the construction of an apparatus[2] which has satisfied these requirements The apparatus was built in 1937 at the University Clinic of Oslo It is transportable and of the size of a regular electrocardiograph It is stable and ready for use wherever a high-frequency alternating current is available (Fig 6) It has without modification readily lent itself to the automatic recording of progressive processes like the coagulability of the blood, the determination of prothrombin, quantitative estimation

102. Swedin, B.: Untersuchungen ueber den Aggregationsmechanismus der Erythrocyten Stockholm, 1936, Centraltryckeriet, 91 pages.

103. Baldes, E. J., and Nygaard, K. K.: Determination of the Coagulability of the Blood Plasma by the Photo-Electric Cell, Proc. Staff Meet, Mayo Clin. 11: 151-155, 1936

104 Hartmann, W., and Wullstein, H.: Motor Process of Vibrating Labia Vocalia of Laryngeal Preparations Examined by Improved Photoelectric Cell, Arch. f. Ohren-, Nasen- u. Kehlkopfh. 144: 348-360, 1938.

105 Hertzman, A. B.: Photoelectric Plethysmography and Sphygmography in Man and Animal, Am. J. Physiol. 123: 99-100, 1938.

106. Festen, H.: Eine objektive Methode zur Bestimmung der Blutgerinnungszeit und Gerinnungsdauer, Munchen. med. Wchnschr. 2: 1370-1371, 1937.

107. Nygaard, K. K., and Baldes, E. J.. Interpretation and Clinical Significance of Coagulograms in Obstructive Jaundice, Proc. Staff Meet., Mayo Clin. 11: 705-709, 1936.

108. Hertzman, A. B.: Photoelectric Plethysmography of the Nasal Septum in Man, Proc. Soc. Exper. Biol. & Med. 37: 290-292, 1937.

109. Hertzman, A. B: Photoelectric Plethysmography of Fingers and Toes in Man, Ibid 37. 529-534, 1937.

110. Hertzman, A. B.· Comparative Estimation of Blood Supply of Skin Areas From Photoelectrically Recorded Volume of Pulse, Proc. Soc. Exper. Biol. & Med. 38: 562-564, 1938.

111. Hertzman, A. B.: Skin Vascular Reactions to Cold Pressor Test, Ibid. 38· 628-629, 1938

112. Hertzman, A. B.: The Blood Supply of Various Skin Areas as Estimated by the Photoelectric Plethysmograph, Am. J. Physiol. 124: 328-340, 1938.

113. Gross, Franz· Über die Reduktionszeit des Blutes, Ztschr f. d. ges. exper. Med. 102: 766-777, 1938

114. Nygaard, K. K., and Guthe, Th.: Automatic Recording of the Wassermann Reaction by a Photoelectric Principle (Photelgraph), Acta med. Scandinav. 97: 293-306, 1938.

115 Evelyn, K A, Malloy, H T, and Rosen, Charles. The Determination of Ascorbic Acid in Urine With the Photoelectric Colorimeter, J. Biol Chem. 126: 645-654, 1938.

116. Nygaard, K. K: A New Apparatus for Photoelectric Recording of the Coagulability of the Blood and Other Progressing Processes, J. Lab. & Clin. Med. 24: 517-522, 1939.

117. Guthe, Th, and Nygaard, K. K· The Photoelectric Determination (Photelgraph) of Ascorbic Acid, Chem. & Indust 57· 1195-1199, 1938

118. Guthe, Th., and Nygaard, K K. Studies on Ascorbic Acid by Means of the Photelgraph. I General Co [illegible] bility to the Study of [illegible]

119. Guthe, Th., and Nygaard, [illegible] of Reversible Oxidati[illegible] Ibid. 101. 40 61, 1939

120. Nygaard, K. K. Studies on the Velocity of Blood Coagulation by the Photoelectric Principle; With Particular Reference to Prothrombin Determinations, Lancet, in press

121. Nygaard, K K., Gathe, R, and Guthe, Th. Quantitative Determination of Blood Fibrin by the Photoelectric Method, Scandinav Arch. f. Physiol, in press

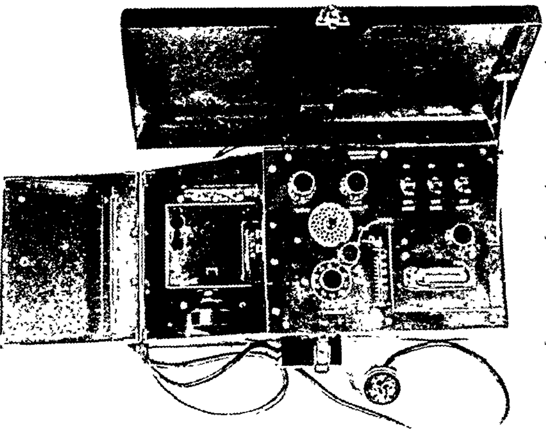


Fig 6 —Photograph of the phototelegraph (open) (Manufactured by I Alnes & Co, Oslo, Norway)

of fibrin, the sedimentation of erythrocytes, and the quantitation of the Wassermann reaction. After ample modifications it has been employed also in the quantitative estimation of ascorbic acid. Having been convinced by these investigations that the apparatus is applicable to a variety of progressive reactions, I have proposed to term the apparatus *the photelgraph.*

The apparatus may be considered to be made up of two main parts, the registering and the recording systems.

The former deals with registrations of variations of light occurring during the process under investigation. It consists chiefly of a constant source of light, a water bath, a socket for the absorption cell containing the specimen under observation, and a photo-electric cell.

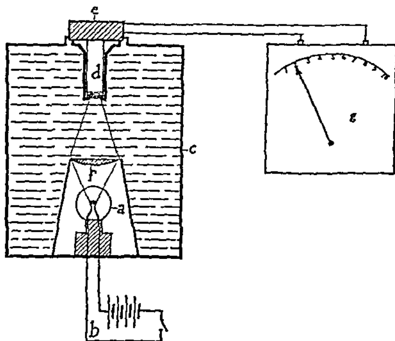


Fig. 5.—Diagram of the coagelometer *a*, 32 candle power automobile headlight bulb; *b*, 6 volt storage battery; *c*, water bath, *d*, Kahn tube, *e*, photronic cell, *f*, lens; and *g*, microammeter.

The latter system deals with a graphic recording of the variations in transmitted light as registered by the photo-electric cell. This part consists of an amperemeter, whose mirror reflects a beam of light onto light-sensitive paper, rotated by a motor at a constant speed per unit of time. In order to obtain satisfactory recordings, ample consideration has been given to the necessity of obtaining a recording system, built into a darkroom compartment which excludes all stray beams of light A diagrammatic sketch of the apparatus is given in Fig. 7.

Source of Light.—This consists of a 35 watt automobile headlight bulb (bilux). It is placed behind and outside the water bath. Storage batteries, 6 volt, of large capacity have served as source of energy.

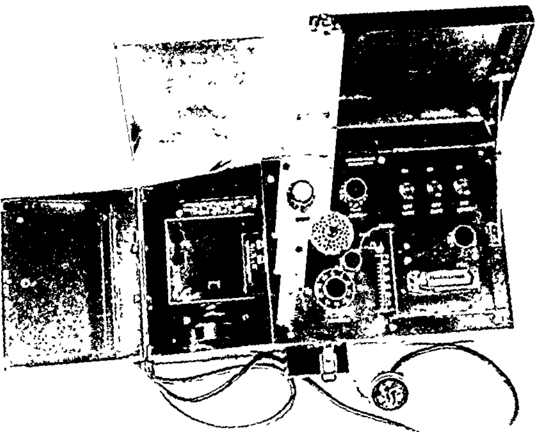


Fig 6.—Photograph of the phototelegraph (open) (Manufactured by J. Alnes & Co., Oslo, Norway.)

The Water Bath.—For studies of coagulability of the blood, the water bath is kept at the constant temperature of 37.5° C. by thermostatic arrangements In the lid of the water bath is a rectangular opening, corresponding to a socket in the water bath and intended to hold the absorption cells of identical form. Through a double convex lens in the back wall and a window in the front wall of the water bath, parallel light traverses in horizontal direction the water bath as well as the specimen contained in the absorption cell.

The Absorption Cells (Test Tubes).—These are specially designed with plane parallel, polished sides. Internally they measure 0.5 by 1 0 cm. and are 5.5 cm. in height. The absorption cells must be optically identical and have to be handled with care to avoid scratching their surfaces. They are inserted in the socket in the water bath with their broadest side exposed to the light. When

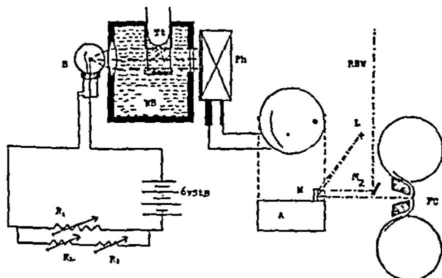


Fig 7.—Dia[illegible] resistances, B, [illegible] cell in position; [illegible] light from bulb [illegible] RBW, vertically [illegible] casette.

in position, exactly 1 sq. cm. of its surface is exposed to illumination. The absorption cells do not stand boiling. When used for studies of coagulability of the blood, they are rinsed with alcohol and ether and left submerged in cold water for a couple of hours, after which they are dried.

The Photo-Electric Cell.—This is of the blocking layer type (Weston Model 594) It is plugged in a socket and located vertically in front of the water bath.

Control of Illumination.—Microrheostats and macrorheostats with a double set of switch contacts are inserted in the current circuit for ample control of the illumination Constancy of illumination is a prerequisite for comparable investigations Control observations of the illumination, which are

performed before and after each test, are readily made by noting the location of a beam of light reflected from amperemeter mirror onto an observation window in the lid of the darkroom compartment.

In order to exclude possible interferences from stray light in the laboratory during the performance of the tests, the registering system here dealt with is covered with an individual lid. In Fig. 6 this lid is seen opened.

The Amperemeter.—This is a rebuilt Weston microammeter (Model 600) with a capacity of 100 microamperes and a sensitivity of 10^{-6}. Its needle is replaced by a small mirror which with its longest axis is placed vertically in the center of its movements.

By a special technical arrangement, the whole amperemeter can be made to rotate horizontally about the axis of the vertical mirror. This arrangement has been chosen in order to facilitate the recording of processes in which an extensive variation of illumination during the period of observation otherwise would have caused the recording beam of light to fall outside the photosensitive paper.

Film.—Photosensitive paper of the perforated type, 6 cm. wide, as used in electrocardiography, has been employed. It is included in the film casette containing a feeder and a receiver of the photosensitive paper, which is rotated at a constant speed per unit of time by two synchronous clock motors. Connected with the motors is a light cutter arrangement which enables an automatic transference of units of time onto the photosensitive paper. This is placed 18 cm. from the mirror of the amperemeter, thus permitting a sensitive recording.

The film casette is a removable part of the darkroom compartment. After completion of the day's observations, the casette may be removed without danger of exposure of the photosensitive paper and may be taken to a separate darkroom for regular development and fixation.

Darkroom Compartment.—For successful recording, a satisfactory darkroom compartment in the apparatus is essential. It contains mainly the galvanometer and the casette with the rotating photosensitive paper. From a source of light placed outside the darkroom compartment a fine beam of light is admitted through a small opening in its wall onto the mirror of the amperemeter. From this mirror the beam of light is reflected in a horizontal direction onto the recording paper. In front of the paper a second mirror is placed in such a way as to divide the reflected light from the amperemeter in two parts, the one part going directly toward the photosensitive paper, the second part being reflected vertically onto a specially designed window in the lid of the darkroom compartment The intention of this arrangement is evident It permits the observer to follow visually the deflections of the amperemeter mirror during the actual recording of the process and arrest the recording when the progressing process is completed. As mentioned above, it also permits control observations of the constancy of illumination

During the present study, various investigations have been carried out to determine whether the use of filters would change or improve in any way the

study of the velocity of blood coagulation. It appears that with the present technique filters are not essential. In a subsequent chapter it is further to be shown that quantitative determinations of fibrin by the photo-electric technique may also be performed without the use of filters.

References

1. Baldes, E. J., and Nygaard, K. K.: Determination of the Coagulability of the Blood Plasma by the Photo Electric Cell, Proc. Staff Meet., Mayo Clin. 11: 151-155, 1936.
2. Nygaard, K. K.: A New Apparatus for Photoelectric Recording of the Coagulability of the Blood and Other Progressing Processes, J. Lab. & Clin. Med. 24: 517-522, 1939.

CHAPTER IV

PHYSICAL AND PHYSIOLOGIC CONSIDERATIONS OF THE PHOTELGRAPHIC TRACINGS (COAGELGRAMS)

Interpretation of the Coagelgram

Searching for a meaning of the photo-electrically obtained tracing illustrated in Fig. 8 it will be recalled that the tracing represents nothing more *than a direct recording of variations of transmitted light* obtained during a certain experiment within a definite length of time. The part of the tracing near the top of the film indicates relative maximal illumination of the photo-electric cell, while relative minimal illumination is to be found at its base

From a physical point of view the tracing of Fig. 8 is clear: From the zero point to a point designated by the letter *F*, the relative maximal illumination of the photo cell remains fairly constant Between point *F* and point *C*, there is a gradual decline toward lower values of illumination. Passing point *C* *the tracing for a shorter space of time indicates relative minimal value of illumination* At a point designated by the letter *R*, the tracing again ascends toward higher values of illumination.

With the present experimental setup, it is evident that these relative variations of transmitted light are the direct result of certain changes occurring in the blood plasma during the process of coagulation. These changes have been investigated in the following manner:

Interpretation of Point F.—From a specimen of citrated or oxalated blood plasma *a series of tracings of the process of blood coagulation induced by re*calcification has been obtained under standardized conditions. The time interval from the beginning of the reaction to point *F* is noted. From the same *sample of blood plasma, another series of observations was performed. Instead* of recording the process of coagulation photo-electrically, however, a fine wire was inserted into the specimen at intervals of 10 seconds. Again the time interval was noted from the beginning of the reaction to the point where the very first fine thread of fibrin appeared on the inserted wire It was found that the first appearance of a fibrin thread coincided with point *F* of the tracings of the identical specimen, as far as time was concerned. (Fig. 9)

It is concluded that *the change of transmitted light during the coagulation of recalcified blood plasma apparent at point F of the photo-electric tracing is the result of the beginning formation of fibrin in the specimen under observation.*

The Transition of the Plasma to a Gel.—It is a well-known observation *that following the first formation of fibrin* threads the recalcified plasma is transformed only gradually from a sol to a gel. This is identical to the gradual onset of clotting of whole blood in vitro. The present experimental arrangement permits a recording *and study of this transformation of the plasma.* It

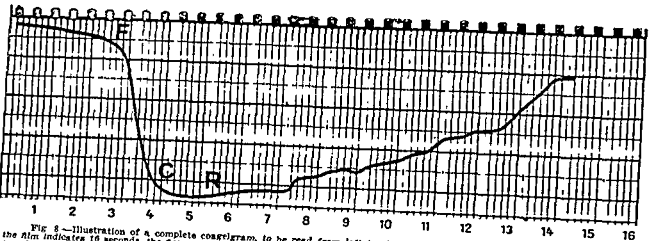


Fig 8.—Illustration of a complete coagelgram, *to be read from left to right.* The interval between each vertical line on *the film indicates 10 seconds*, the figures at the bottom of the film indicate minutes. *F* designates first formation of fibrin; *C*, completion of fibrin formation, and *R*, beginning of clot retraction. The complete process of coagulation is thereby divided into four phases, as indicated in the text.

In subsequent illustrations, when interest is mainly centered on the first *two phases of* the process, only incomplete coagelgrams are included, that *is, tracings concerning only* the first two phases of the process. For the sake of convenience the lettering of points *F* and *C* is omitted where not necessary for the further understanding of the coagelgrams.

may here be pointed out how this study is simplified by a certain technical arrangement of the apparatus employed. Through the previously described window in the lid of the apparatus it is possible to follow indirectly the progression of the process of coagulation. This naturally also permits an interruption of the recording and the simultaneous visual observation of the specimen under investigation at any point during the progression of the reaction.

Fig. 10 illustrates a series of photo-electrically obtained tracings of samples of recalcified plasma taken from the same specimen. The tracings of Samples *b* and *c* were obtained by interrupting the recording at various intervals past point *F*. With the interruption of the recording the sample under

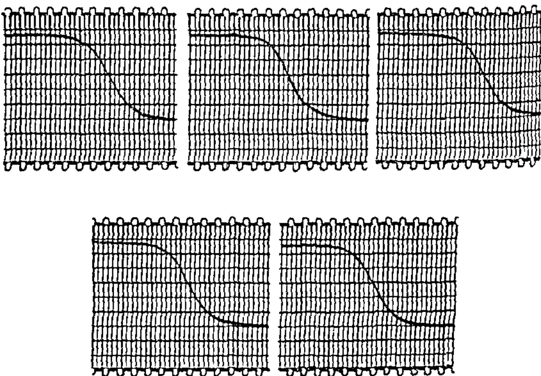


Fig 9—A series of five coagelgrams from the same specimen. The results of these coagelgrams to be correlated with the results obtained by mechanical means as described in the text.

SAMPLE NUMBER	REMOVAL FIRST FIBRIN THREAD AFTER INTERVALS (SECONDS)
6	130
7	120
8	115
9	100
10	110

investigation is observed visually, in order to determine whether the plasma is still in a liquid state or clotted. In this particular figure the Sample *b* at the point of interruption of the process was still in a semiliquid state. As indicated in the recording of Sample *c*, only a few additional seconds sufficed to transform the plasma into a gel In this particular example the transition of the plasma from a sol to a gel required about 30 to 40 seconds after the first formation of

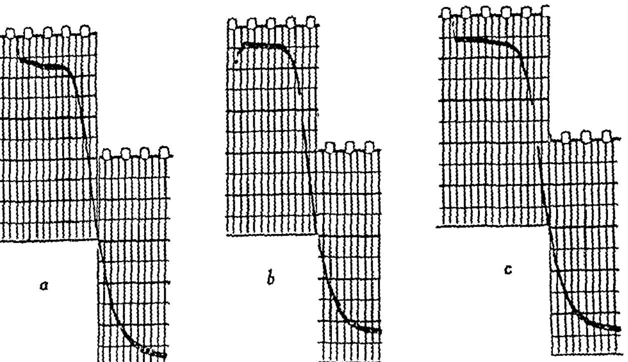


Fig 10—A series of three coagelgrams from same specimen *indicating* the sequence of events during coagulation: *a*, noninterrupted observation, *b* and *c*, observations *interrupted* at *varying* points past *F*. At the time of *interruption* specimen *c* was transformed to a gel, while *b* was still semiliquid. The period from *F* to complete gel formation as in *a* indicates the time required for *transition* of *specimen* to a gel after first formation of fibrin. Vertical linear distance from point *F* to point of tracing coinciding with moment of completed gel formation *indicates* relative quantity of fibrin formed up to this point.

fibrin threads. The duration of this particular stage of the coagulation of plasma is only relative. Generally it may be stated that this period stands in a certain relation to the duration of the process previous to the formation of fibrin

Interpretation of Point C.—Referring to the tracings of Fig. 10c, it may be noted that after the formation of a gel the tracing continues its gradual decline to point *C*, where it levels off. This indicates that following the formation of a gel something occurs in the specimen resulting in a continuation of the reduction of transmitted light.

The possibility has been considered that this reduction is effected by a retraction of the clot following directly on the transition of the specimen to a clot. This hypothetic retraction would then necessarily occur without any expression of serum, as a combination of retraction and expression of serum would result in a relative increase of transillumination. From the uniformly smooth appearance of the tracing between points *F* and *C* it is not very likely that the reduction of transmitted light is the result of two phenomena of such fundamentally different nature as formation of fibrin and retraction of the clot. In addition, it is well known from hematologic literature that blood specimens from patients with certain hemorrhagic diseases, such as thrombocytopenic purpura or aplastic anemia, do not exhibit any retractility of the clot. Recordings of the coagulability of blood plasma from patients with these diseases do not, as related to the decline of the tracing between points *F* and *C*, differ in type from samples exhibiting retraction of the clot.

Judging from the smooth appearance of the tracing, it seems reasonable to believe that no fundamental difference exists between the process producing the first part of the decline of the tracing and that of the latter part of this phase

This latter possibility has been investigated in the following manner: From a specimen of blood plasma a tracing is obtained covering the period of coagulation from the zero point to point *C*. In subsequent observations of samples from the same specimen, the recording of the process has been interrupted at points gradually approaching point *C*, but past the point of gel formation. By inserting at these points a fine wire into the specimen, the entire clot is rapidly removed, leaving a clear liquid in the cuvette The cuvette is now again quickly inserted into the water bath and the recording continued.

A demonstration of the result of this study is illustrated in Fig. 11 From Fig 11 *c* and *d*, it is noted that the continued recording exhibits evidence of further reduction of transmitted light after mechanical removal of all of the fibrin formed up to the time of interruption of the process By visual observation following the completed recording it is found that the content of the absorption cell is still completely transparent and clear. By turning the cuvette upside down, it is with some surprise one notes that the clear content is not liquid but completely clotted. By again inserting a fine wire, a veil of fibrin is removed Following this last removal of fibrin the remaining liquid has been observed for hours without any further appearance of clot formation, indicating that this fluid is serum.

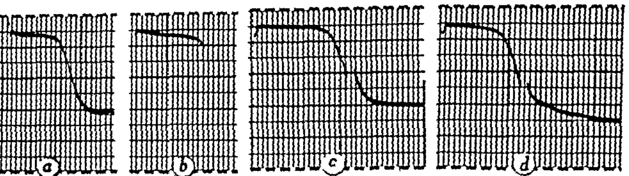


Fig 11.—A series of four coagelgrams from same specimen indicating the sequence of events during coagulation. *a*, noninterrupted observation, *b*, at the moment of interruption of the recording the sample was still liquid; *c*, at moment of interruption the sample was clotted *d*, at moment of interruption the fibrin already formed was mechanically removed The continued recording indicates further formation of fibrin, thus illustrating that fibrin is formed up to a point closely approaching point *C* NOTE In order to record the formation of minute quantities of fibrin after the interrupted observation in *d*, the further recording was undertaken with a greatly increased illumination. This explains the peculiarity of the end of tracing *d* as compared to the others.

From these observations it is concluded that, whereas the beginning of the first formation of fibrin coincides with point *F* of the tracing, *the formation of fibrin is completed at point C. The reduction of transillumination between points F and C is the result of the continuous formation of fibrin.*

Referring again to Fig. 10 and judging from the moderate reduction of transmitted light during the transition of the plasma from a sol to a gel, it is apparent that only a moderate amount of the totally available fibrin suffices for the formation of a gel. This finding is clearly substantiated by the investigations demonstrated in Fig. 11*d*. These recordings, as well as the visual observations mentioned earlier, indicate that formation of fibrin scant enough not to alter materially the clear watery appearance of the specimen still suffices for the formation of the specimen into a clot. It appears that one would expect a very minute quantity of fibrinogen before a gel formation is made impossible. It certainly seems as if nature also in this respect has provided for an abundance of material for clot formation. It appears reasonable to assume that this point explains the relatively few true cases of so-called fibrinopenia recorded in the literature.

For a variable length of time the tracing of the process continues horizontally past point *C*, indicating that during this phase of the reaction changes affecting the transmitted light do not occur in the sample.

Interpretation of Point R.—Past point *R* of the tracing the upward trend of the tracing signifies that increasing illumination is reaching the photo-electric cell. The appearance of this part of the tracing, in contrast to the regular appearance of the previous phases of the process, is found to be irregular in every way, even in samples from the same specimen. The interpretation of this phase of the reaction is clear. It is observed to be the result of the retraction of the clot with expression of serum. This phase completes the process of coagulation of the blood. Knowing that this last phase of the process is influenced by partly known, partly unknown hematologic as well as physical factors, one easily realizes that significant tracings of the clot retraction can hardly be achieved without particular consideration of these factors. A special consideration of this problem will not be taken up in the present work.

Using a similar experimental apparatus, Baldes obtained an identical type of tracing during the spontaneous coagulation of whole blood.

Ultramicroscopic Observations and Photography.—The investigations here carried out for the interpretation of the coagelgrams have been supplemented by direct ultramicroscopic study of oxalated or citrated plasma brought to coagulation by recalcification, a type of observation employed by several workers.

A description of the ultramicroscope used for this study is considered of no particular interest, as it is of the type regularly in use in any laboratory for the ultramicroscopic study of, for example, *Treponema pallidum*.

As regards technique, it may be mentioned that the recalcification of the plasma is undertaken in the regularly employed absorption cells in the usual manner From the absorption cell a drop is transferred to the slide with a fine pipette and then dealt with as for regular ultramicroscopic observation. It soon

became clear that oxalated plasma was less suitable than citrated plasma for this type of investigation as the precipitated calcium oxalate crystals sedimented, causing an abundance of reflected light to interfere with the closer study of details. Consequently, only citrated plasma was used. Another point of interest became evident. By this ultramicroscopic arrangement a direct parallelism as to time between the period of fibrin formation of the coagelgrams and the identical period as judged from the ultramicroscopic investigation could not be obtained. This apparent discrepancy may be explained by the fact that the ultramicroscopic apparatus did not include technical facilities for temperature control. It must also be recalled that the relation between the volume of the specimen and its glass-exposed surface is entirely different in the ultramicroscopic study as compared to the specimen contained in the absorption cell during photo-electric investigations Even when a direct substantiation of our interpretation of the coagelgram could not be obtained by ultramicroscopic studies, certain observations seem significant enough to be discussed in this connection.

By ultramicroscopic investigation of a nonrecalcified citrated plasma it may be noted that the specimen consists of a varying number of platelets and hematoconiae scattered throughout an otherwise clear visual field

In a specimen of recalcified citrated plasma the appearance is practically the same from the onset of the observation After a time, however, a noticeable change gradually takes place in the appearance of the platelets. At the beginning of the observation the platelets appear of fairly smooth outline with an occasional small pseudopodia-like extension. As the process proceeds the outline of the platelets assumes a more ragged, uneven appearance. At the same time they give the impression of swelling slightly. At this stage two or more platelets may be seen to clump together, thereby losing their integrity.

The observations here related are so uniform from one observation to another that the worker has the definite impression that these visual changes are a significant, integral part of the progressive reaction of blood coagulation. This impression is strengthened by the observation that these changes are more marked in fast coagulating specimens, in sharp contrast to the very slowly coagulating specimens, such as those of hemophilia.

These observations can be directly related to a peculiarity of the coagelgram during this first phase of the process As may have been noted, the tracings before reaching point F are usually not quite horizontal but indicate minute reductions of transillumination. After our ultramicroscopic studies, it was concluded that these minute reductions of transillumination are brought about by the heretofore described transformation and clumping of the platelets.

Let us now return to a further description of the progress of the process as viewed through the ultramicroscope. After the initial and gradual changes in the platelets, no new morphologic changes may be observed for a time. Then, suddenly, interesting things start to occur Somewhere in the clear field, apparently out of nowhere, has suddenly appeared a small refractile needle, smooth in appearance, invariably straight, frequently with sharpened ends. In

the next moment crops of similar formations may be shooting up everywhere, just as frequently in the clear field as close to the clumps of platelets. The needles may each vary slightly in size and thickness, may cross each other in all directions, and after a time may be focused throughout the layers of the specimen, giving the field a weblike appearance considered characteristic of the often mentioned fibrin web or mesh. (Fig. 12.)

In specimens in which the coagulation time is prolonged, as evident from the coagelgram, the appearance of the fibrin needles is so protracted that one would think it possible by the present ultramicroscopic investigations to obtain a clear conception of the details of the formation of these needles In this I have not succeeded. When the needles suddenly appear they are in their final shape without any appreciable subsequent changes. There appears to be no particular place for their formation, as they are seen to appear just as frequently in a clear field as close to the platelets, discrete, or in clumps. I have not been able to confirm the statement occasionally noted that the fibrin needles originate from or are centered around the platelets or platelet clumps, which usually have been considered a center of coagulation.

In quickly coagulating specimens the appearance of the needles may occur so rapidly that the observer has the impression that a fine net is suddenly thrown across the microscopic field.

After the fibrin web is formed, the further development of this is morphologically less conspicuous and clear. The hematoconiae present from the beginning and in constant Brownian movement continue their motions a long time after formation of the fibrin web. The latter slowly becomes more and more substantial; that is, being extended to all layers of the specimen. After a time, varying with the coagulation time of the specimen, the hematoconiae gradually slow up their movement until finally they are arrested between the masks of the fibrin web.

From the morphologic study of the coagulation of recalcified citrated plasma by the ultramicroscope, *it is concluded that the process of coagulation occurs in two well-defined phases: (1) an introductory stage with changes in the size and outline of the platelets with a tendency to clumping of these corpuscular elements; and (2) a second stage with sudden appearance of a few or many fibrin needles gradually increasing in number with varying rapidity, thereby forming a characteristic fibrin web.*

So far as the time element is concerned, the present ultramicroscopic investigation cannot be applied directly to the time indication of the identical phases of the process as evident from the coagelgrams, mainly because of incongruence of physical factors by the two types of investigation (temperature re- [illegible] how- [illegible]

during [illegible]

As far as biologic phenomena are concerned, the ultramicroscopic investigations have given a direct demonstration of the sudden appearance of fibrin needles, growing in number with varying degrees of rapidity, thereby present-



Fig. 12.—The ultramicroscopic appearance of the fibrin web in a specimen of recalcified citrated plasma.

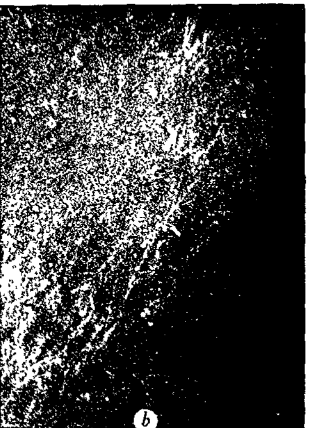




Fig 13—The ultramicroscopic appearance of the progressive changes in a coagulating specimen of recalcified citrated plasma. Note (*a* to *b*) *the practically clear field* at the start of the process. During the progression of coagulation the field is gradually obscured by a fibrin web of increasing density.





Fig 13 continued

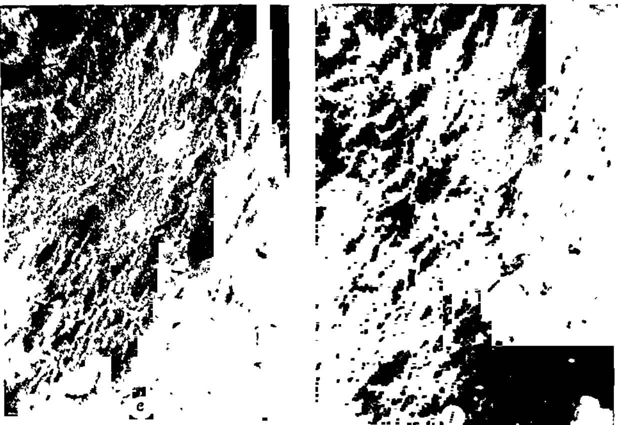


Fig. 13 continued

ing the morphologic basis for a physical understanding of point *F* and the subsequent course of the photo-electrically obtained tracing of the process of coagulation.

Finally a correlation is obtained between the appearance of the coagelgrams and the rapid or slow formation of fibrin in various blood samples. In specimens in which there occurs a prolonged period before the occurrence of the formation of fibrin as seen through the ultramicroscope, the further formation of a more substantial fibrin web is prolonged in relation to a more quickly reacting sample. Specimens with prolongation of the coagulation time as apparent from the flattened appearance of the coagelgrams reveal, by ultramicroscopic observations, a slow formation of the fibrin web as compared to normal specimens.

It may further be mentioned that the ultramicroscopic investigations performed explain a peculiarity observed during the work with the interpretation *of the coagelgrams by the help of the fine wire inserted intermittently into the* specimens In rapidly reacting samples the inserted wire, at the first appearance of fibrin needles hanging onto it, would be able to remove a large clot almost immediately afterward In specimens reacting slowly, as those from cases of hemophilia, small clumps of fibrin could be removed from time to time for several minutes from the liquid specimen before the removal of a real clot and gelling of the specimen

From a physical point of view it ought to be mentioned that the photoelectric cell cannot be expected to register the very first appearance of a single fibrin needle From the observations here carried out it appears, however, that in all cases few or many needles simultaneously appear, explaining in every case the reason for the more or less conspicuous point *F* of the tracing. The natural deduction of this observation is that point *F* in all cases is more or less protracted to the very first appearance of the first fibrin needle On physical grounds, however, the relation between the formation of fibrin and point *F* of the tracing must be considered maintained, as the photo cell in all cases will start to register the same minimal number of fibrin needles With this reservation the expression is maintained throughout this work that point *F* indicates the beginning of fibrin formation, while there is actually a slight lag period before such a registration is apparent from the coagelgram

With the intention of conveying a direct visual impression of the main points of these ultramicroscopic studies, attempts were made to photograph the ultramicroscopic observations with the help of a 16 mm kodak motion picture camera The illustration in Fig 13 is an example of the results obtained. In Fig 13 a reproduction is presented of various stages of the coagulation of recalcified citrated plasma In this particular experiment the motion picture camera was run at the speed of eight exposures per second

Summarizing the observations here stated, it may briefly be concluded that *with the present photo-electric recording of the process of coagulation of the recalcified plasma, certain points of the tracing are produced by and coincide with certain fundamental biologic changes in the specimen under observation Point F of the tracing coincides with the first formation of fibrin. Between*

points F and C a continuous formation of fibrin occurs. Clot retraction, with expression of serum, begins at point R.

Four Phases of Blood Coagulation.—As a result of the foregoing data it is evident that the coagelgram, on the basis of its geometric appearance by the points *F*, *C*, and *R*, may be divided into four parts representing four different phases of the complete process of blood coagulation. Consequently the coagelgram may be said to represent an indirect, continuous recording of the process of coagulation with its sequence of main events. This automatic division of the process on the basis of the *geometric appearance of the coagelgram appears to* be in accord with the general conception of the phenomenon of blood coagulation as presented by previous and present workers concerned with the physiology of this process.

To simplify the further exposition of this work, it is deemed advisable to designate these various phases by certain terms. In selecting a terminology, it is considered desirable to use terms which are used in the current literature and which in no way commit the present investigation to one or the other of the many varying conceptions of the complex physiology of the coagulation of the blood. For reasons to be touched upon later, the following terminology has been employed. It includes, with slight modifications, the terms used among others by Hammarsten,[1] Fonio,[2] Pickering,[3] and Mills:[4]

1. *Period of dissociation,* comprising the interval from the beginning of the reaction to the first formation of fibrin.

2. *Period of fibrin formation,* comprising the entire, continuous process between points *F* and *C* of the coagelgram.

3. *The rest period,* between points *C* and *R*.

4. *Period of clot retraction,* beginning at point *R*

Aside from the variability of the duration of the four periods, the coagelgram *introduces a fifth variable; namely, the extent of the relative reduction of* transmitted light during the second phase of the process. On physical grounds and with the reservation that the foregoing statements can be substantiated, it may be inferred directly that the degree of relative reduction of transmitted light during the period of fibrin formation under standardized conditions is an expression of the quantity of fibrin present in the specimen. This inference is the origin of a quantitative method for the photo-electric determination of fibrin, to be taken up in a later chapter. A substantiation of certain points of the interpretation here presented regarding the second phase of the process is thus obtained.

The illustrations of the coagelgrams presented in this chapter have all been obtained by using recalcified citrated plasma. Recalcified oxalated plasma may be used without changing the essential appearance of the coagelgram. The only variation noted is a moderate rapid reduction of the transmitted light at the very beginning of the period of dissociation. This is explained as a result of an initial precipitation of calcium oxalate crystals. It is readily understood

that this phenomenon may complicate the initial adjustment of illumination to a fixed standard value in the individual specimens where such a standardization is considered important.

Comment

During the present study I have realized the main potentialities of the present investigative technique as lying in the field of analytic physiology. Not being a physiologist myself, these potentialities consequently had to be curbed in essential points. The present investigation in no way intended to probe into the perplexing problem of the physiology of coagulation of the blood For certain reasons, however, it seems desirable to touch upon a few of the less questionable points of this process, points which have a more direct bearing on the findings and the interpretation given in the foregoing.

There is general agreement among the numerous workers concerned with the physiology of blood coagulation that the formation of fibrin and the subsequent transition to a gel is the result of an interaction of fibrinogen and thrombin. The thrombin does not exist in the blood per se but is considered to be the product of prothrombin (thrombogen, serozyme) and a coagulant substance contained in blood and tissue cells, thrombokinase (thrombozyme, cytozyme). This interaction between prothrombin and thrombokinase is proved to take place only in the presence of sufficient amounts of active calcium ions Some investigators consider prothrombin to be present in the circulating blood. Others present the idea that only an inactive form, as its precursor, is free in the blood (proserozyme of Bordet) and in unknown ways transformed into its active form (serozyme of Bordet). The coagulant material obtained from cell substances has been proved to contain a lipoid, cephalin (Howell). It is, however, considered most likely that this substance is not quite identical to the so-called thrombokinase which, according to Howell, is a water-soluble, thermolabile complex of cephalin proteins exceeding to a considerable degree the coagulant effect of pure cephalin

The nature of the prothrombin-calcium-thrombokinase reaction into the formation of fibrin is still considered unknown. Of interest for the present investigation is Mills'[14] conception that thrombin appears to be formed only a few seconds before the first formation of fibrin. This agrees with the original concept of Hammarsten,[1] who distinguished between two main events in the physiologic process of blood coagulation, namely, (1) the formation of thrombin and (2) the transition of fibrinogen into fibrin.

The first of these two phases seems to be comparable to the first period illustrated in the coagelgrams, if not identical. Whether or not this is also identical to the period of thrombin formation will not be considered here. This is a point to be remembered in attaching a term to this first phase of the process.

The following line of reasoning has presented the lead Based on the coagelgrams of numerous observations from normal and abnormal cases it is found that, according to the duration of the first period of the process of

coagulation, observations may be divided into three main groups: (1) normal, (2) those *with shortened*, and (3) *those with prolonged first period* as compared to the normal period. Be it in native or in oxalated or citrated form, the plasma may be considered in all these cases to possess a certain stability whereby its liquid state is retained. Breaking this stability in one way or another results in the release of a factor or factors of still unknown nature, initiating the process of coagulation. The duration of the first period, until the appearance of fibrin, may be taken as an expression of the original degree of stability of the plasma. This expression may be of comparable value under standardized conditions. The principle that breaks the stability of the plasma may be of chemical or physical nature (calcium ions in the case of salted plasma or glass surfaces in the case of native plasma). The total number of processes of whatever nature they finally may prove to be may be considered active in breaking down or dissociating the stability of the plasma. Mills has also demonstrated that this dissociation occurs up to a point immediately prior to the formation of fibrin, as the progressive process may be arrested by adequate measures at this very point. For these reasons I have considered it justified to apply the term *dissociation period* to this first phase of the process of blood coagulation.

During the second phase, fibrin formation is found to take place during the entire period. The terms applied to this and the last two phases of the process are consequently purely descriptive.

Controversies still exist as regards the nature of the thrombin-fibrinogen reaction. It has been considered a fermentative process, a purely chemical as well as a colloidal-chemical reaction, and a process of crystallization. A review of this fundamental problem is considered to fall outside the scope of the present investigation. When the possibilities of the present technique with regard to the more obscure physiologic problems of the process of blood coagulation are considered, I anticipate that further study of these problems may be of importance. As is directly obvious from the appearance of the tracings and from the preceding interpretation, the coagelgram, during the formation of fibrin, represents the velocity curve of this reaction Coupled with subsequent information regarding the relation of the quantitative formation of fibrin to the geometric appearance of the curve, sufficient data appear to be at hand for the mathematical treatment of this velocity curve, thereby furnishing a possible approach to the understanding of the biologic nature of the thrombin-fibrinogen reaction

Without this aid certain points are of consequence for the conception of this second stage of the process

The progressive reaction of coagulation of recalcified plasma can be arrested by the addition of anticoagulant solution at points during the process even past the formation of some fibrin. In order to obtain a mixture of the anticoagulant solution and the coagulating plasma, it is obvious that the addition must be undertaken before the transition of the specimen to a gel. The fibrin already formed will then be found floating in a liquid specimen which

does not proceed to gel formation. In this connection it may be recalled that Hammarsten found that a solution of fibrinogen can be coagulated by thrombin without the presence of calcium. He concluded that calcium was active only during the first phase of the coagulation but was not concerned with the formation of fibrin. During later years suggestive evidence has indicated that thrombin may prove to be a colloidal calcium-cephalin compound (Ferguson[4]). This controversial point appears to be of no direct consequence in interpreting the above observations of arrest of the process during the early stages of the second phase. It is a general finding that thrombin added to oxalated or citrated plasma results in rapid formation of fibrin. Eagle[6] did not succeed in inactivating thrombin by the addition of an oxalate solution.

The logical assumption would be that the anticoagulant solution when added to recalcified plasma after the onset of fibrin formation exerts an anticoagulant activity identical to that in whole blood; that is, through inactivation of the calcium ions, thereby preventing the development of the thrombokinase-prothrombin-calcium reaction which otherwise would go on to the formation of thrombin. In other words, it appears that the formation of thrombin is progressing also after the onset of fibrin formation

It is a well-known fact that in a certain period after coagulation the serum contains considerable quantities of thrombin. Arthus[7] (1902) estimated the coagulant activity and the concentration of thrombin on a solution of fluoride plasma. He concluded that the thrombin formed immediately before the actual formation of fibrin represents only a small fraction of the quantities present after completion of coagulation; further, that there is a continuous production of thrombin during coagulation and actually continuing after the completion of gel formation.

As to the nature of the thrombin-fibrinogen reaction, certain findings seem difficult to reconcile with the prevalent opinion that the reaction is fermentative. The following investigations are mentioned as they appear to be of some consequence for the present exposition of the problem

According to Rettger's[8] investigation, a quantitative relation exists between the thrombin and the fibrinogen. Submaximal quantities of thrombin effect only partial fibrin formation when added to surplus of fibrinogen, the rest of the fibrinogen being rapidly transformed to fibrin upon the addition of more thrombin. Howell[9] continued his pupil's investigations and found the reaction to be only partly quantitative. Increasing the amount of thrombin resulted in the formation of increasing quantities of fibrin in the relation of 1:215. With increasing quantities of fibrinogen, however, this relation was slightly altered, presumably because of mechanical absorption of thrombin by the fibrin already formed

These investigations by Rettger and by Howell have been criticized by Eagle,[6] who argued that with small concentrations of thrombin the coagulation occurs so slowly that the equilibrium is very difficult to define and therefore to be regarded as a very poor basis for quantitative conclusions. In the same paper Eagle concluded that thrombin does not disappear from a mixture of

thrombin and fibrinogen until the moment of coagulation and from that point on it disappears in large quantities. It appears that this observation constitutes no valid objection to the conception of Arthus, as Eagle in these studies worked with a preformed solution of thrombin.

The preceding paragraphs have been included to indicate that a presentation of the sequence of the main events of blood coagulation is linked with the fundamental, still unsettled problems of the physiology of blood coagulation A division into its main events is therefore to be presented with some reservation. The possibility has been pointed out that *Hammarsten's original conception of the process as falling into two well-defined phases, that of thrombin and that of fibrin formation, may be modified in the respect that physiologically these two phases are well-defined and separate developments of the process, as regards time, however, developing simultaneously. As a consequence the dissociating processes may be considered not too definitely limited to the first phase of the reaction.*

It appears that no practical error is committed by regarding the dissociating processes as limited to the first phase of the reaction It is a uniform finding throughout this investigation that a definite relation exists between the duration of fibrin formation and the preceding first phase of the process. Prolongation of the one is associated with equal prolongation of the other, *while an increased velocity is equally apparent in both stages of the process.* An identical finding is brought out by systems operating with recalcified plasma containing excess of thromboplastin or coagulation of a solution of fibrinogen with pure thrombin preparations.

This interrelation of the two phases of the process is not easily grasped if we retain the idea of the fermentative nature of the reaction together with Hammarsten's concept of two well-defined, separate phases of the formation of thrombin and fibrin. After completion of thrombin formation, one would then expect a fermentative action of thrombin on fibrinogen, resulting in a second phase of rather constant duration, irrespective of the duration of the first phase. Considering thrombin formation as continuing also during the second stage of the process, this time relation between the two stages may readily be understood. The period of dissociation concerns the processes leading to the first liberation of thrombin. The velocity of this process is a relative expression of the activity of these dissociating processes. These reactions, continuing also into the second stage of coagulation, would equally determine the velocity of this stage. It is *concluded that the rate of coagulation may be considered a relative expression of the activity of the processes leading to the liberation of thrombin.*

Reading of the Coagelgrams

In order to be able to employ the coagelgram as a quantitative expression of the coagulability of the blood, it is necessary to deal briefly with the technique of its reading.

After some practice, the geometric appearance of the coagelgram as a whole will convey to the observer an immediate impression of the coagulability of the blood, having the normal coagelgram in mind. During serial observations an impression of the variability of the coagulability may likewise be had in the same way when the coagelgrams are placed one below the other.

For the convenience of an accurate report, different technique is desirable. Here one is confronted again with the problem of choosing convenient end points. This problem is simplified somewhat by the present technique because the decision as to satisfactory end points is not influencing the physiologic process. The tracing records the whole process continuously. When

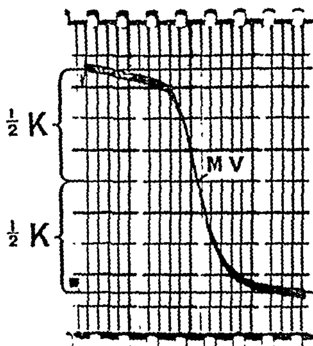


Fig 14—Coagelgram of fast reacting specimen (recalcified citrated plasma) The horizontal line through ½K crosses coagelgram at point of maximal formation of fibrin (point MV).

the observation is completed, we are left with a permanent record of the process, and on the basis of certain characteristics of this recording the question of end point can be decided.

For successful selection of end points in the present method, two main requirements must be fulfilled. (1) the selected point or points must represent identical physiologic events or phases of the coagulation process, in order to assure dependable comparison from case to case, (2) the selected point or points must be geometrically well defined in order to obtain accuracy of readings.

According to the preceding chapter, the duration of the dissociation period and the period of fibrin formation represent quantitative values of identical and well-defined phases of the process

thrombin and fibrinogen until the moment of coagulation and from that point on it disappears in large quantities It appears that this observation constitutes no valid objection to the conception of Arthus, as Eagle in these studies worked with a preformed solution of thrombin.

The preceding paragraphs have been included to indicate that a presentation of the sequence of the main events of blood coagulation is linked with the fundamental, still unsettled problems of the physiology of blood coagulation A division into its main events is therefore to be presented with some reservation. The possibility has been pointed out that *Hammarsten's original conception of the process as falling into two well-defined phases, that of thrombin and that of fibrin formation, may be modified in the respect that physiologically these two phases are well-defined and separate developments of the process, as regards time, however, developing simultaneously. As a consequence the dissociating processes may be considered not too definitely limited to the first phase of the reaction.*

It appears that no practical error is committed by regarding the dissociating processes as limited to the first phase of the reaction. It is a uniform finding throughout this investigation that a definite relation exists between the duration of fibrin formation and the preceding first phase of the process. Prolongation of the one is associated with equal prolongation of the other, while an increased velocity is equally apparent in both stages of the process. An identical finding is brought out by systems operating with recalcified plasma containing excess of thromboplastin or coagulation of a solution of fibrinogen with pure thrombin preparations.

This interrelation of the two phases of the process is not easily grasped if we retain the idea of the fermentative nature of the reaction together with Hammarsten's concept of two well-defined, separate phases of the formation of thrombin and fibrin. After completion of thrombin formation, one would then expect a fermentative action of thrombin on fibrinogen, resulting in a second phase of rather constant duration, irrespective of the duration of the first phase. Considering thrombin formation as continuing also during the second stage of the process, this time relation between the two stages may readily be understood. The period of dissociation concerns the processes leading to the first liberation of thrombin. The velocity of this process is a relative expression of the activity of these dissociating processes. These reactions, continuing also into the second stage of coagulation, would equally determine the velocity of this stage. It is *concluded that the rate of coagulation may be considered a relative expression of the activity of the processes leading to the liberation of thrombin.*

Reading of the Coagelgrams

In order to be able to employ the coagelgram as a quantitative expression of the coagulability of the blood, it is necessary to deal briefly with the technique of its reading

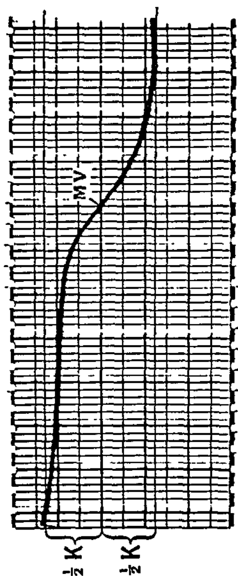


Fig 15 —Coagelgram of slow reacting specimen (recalcified citrated plasma). Point MV is located in same manner as in Fig 14

As indicated earlier and as obvious from Fig. 14, the points *F* and *C* are geometrically well defined only in rapidly reacting specimens, due to the fact that physiologically the onset as well as the completion of fibrin formation is not a sudden but a gradual process. For this reason, I have considered the determination of the dissociation period and the period of fibrin formation as not quite satisfactory as a basis for comparable investigations. Admittedly we have thereby given up the two best physiologic phases of the process.

Let us again study the appearance of the coagelgrams. If the relative reduction of transmitted light during the period of fibrin formation had been constant per unit of time, the tracing between points *F* and *C* would have taken the appearance of a straight line. The slight curve of the tracing during the first half of this second phase indicates increasing reduction of transmitted light per unit of time up to a maximum, following which there is a gradual decrease of reduction. The coagelgram is given an appearance characteristic of the sigmoid curve. The tracing during the second phase constitutes the velocity curve of fibrin formation. *The point of maximal velocity of fibrin formation* coincides with the point of maximal relative reduction of transmitted light during this phase.

From a physiologic point of view, the point of maximal velocity of fibrin formation may be regarded as a rather unusual expression. The present technique, however, readily demonstrates this uniform variation in the rate of fibrin formation. Even if this expression is acceptable only on a theoretic basis, therefore, this point may be considered well defined with the present investigative apparatus It retains its relative position to points *F* and *C* irrespective of the duration of the period of fibrin formation and consequently may form a practical basis for comparable investigations.

The question now arises, how to localize this point. In specimens with a not too flattened coagelgram, our end point may be localized fairly accurately by noting the sharpest angle formed between the tracing during the second phase of the process and the vertical lines of the coagelgram indicating unit of time.

In cases with a prolonged second phase this procedure will not suffice, as it may approach a straight line more and more during this second phase.

In all cases, therefore, I have proceeded as follows: A horizontal line is drawn through the points indicating relative maximal and minimal illumination; that is, through the onset of the tracing in the first period, through the lowest point of the tracing after completion of the second period Let us term the vertical linear distance between these two horizontal lines *K*. A horizontal line through the point ½*K* crosses the tracing at the point indicating maximal velocity of fibrin formation. *This point is designated by the letters MV.* The time relation of this point is read directly off the coagelgram With our present arrangement the distance between each vertical line of the coagelgram indicates 10 seconds A point located between two vertical lines may more accurately be determined by interpolation if this is deemed desirable.

phase of the process leading to the first formation of thrombin. The period of fibrin formation would cover only part of the period of thrombin formation, as thrombin is presumably formed also after all the fibrinogen has been converted.

The velocity of the process of blood coagulation is a relative expression of the velocity of thrombin formation.

It is suggested that the coagelgram, representing a graphic expression of the entire process of coagulation as well as the velocity curve of the fibrin formation, may form an adequate basis for a new physiologic approach to the study of the process of thrombin-fibrinogen interaction.

References

1. Hammarsten, Olof. Ueber die Bedeutung der löslichen Kalksalze fur die Faserstoffgerinnung, Ztschr. f. physiol. Chem. 22: 333-395, 1896.
2. Fonio, A.: Die Gerinnung des Blutes. In Bethe, A., von Bergmann, G, Embden, G and Ellinger, A.: Handbuch der normalen und pathologischen Physiologie mit Berucksichtigung der experimentellen Pharmakologie, Berlin, 1928, Julius Springer, vol. 6, pp. 307-400.
3. Pickering, J. W.: The Blood Plasma in Health and Disease, New York, 1928, The Macmillan Co, 247 pages.
4. Mills, C. A.: The "Period of Induction" in Blood Clotting or the Sequence of Events Preceding Fibrin Formation, Chinese J. Physiol. 1: 123 142, 1927.
5. Ferguson, J. H.: An Intermediary Calcium Complex in Blood Coagulation, Am. J. Physiol. 119. 755-762, 1937.
6. Eagle, Harry: Studies on Blood Coagulation. II. The Formation of Fibrin From Thrombin and Fibrinogen, J. Gen. Physiol. 18: 547-555, 1935.
7. Arthus, Maurice Un réactif quantitatif du fibrinferment; application à l'étude de la vitesse de la production du fibrinferment dans le sang extrait des vaisseaux, J. de physiol. et path. gén. 4: 1-11, 1902.
8. Rettger, L. J.. The Coagulation of Blood, Am. J. Physiol. 24: 406-435, 1909
9. Howell, W. H: The Preparation and Properties of Thrombin, Together With Observations of Antithrombin and Prothrombin, Ibid 26: 453 473, 1910.

In this chapter we have employed a fixed, arbitrarily chosen value of maximal illumination. In a subsequent chapter an exposition is given of the effect of variation in the illumination as regards the appearance of the coagelgrams (see page 98 and Fig. 22). By varying the illumination with all the other factors being constant, it is noted that the duration of the second phase of the process remains unchanged, as is to be expected. The relative value of $\frac{1}{2}K$ is likewise maintained. The point *MV* thereby retains its position as regards the period of time. (Fig. 15.)

Unless otherwise stated, I am subsequently employing as the end point this point of maximal velocity of the formation of fibrin. The time period from the beginning of the reaction to this point I have designated by the conventional term *coagulation time of the blood.*

Summary

An interpretation has been presented of the photo-electric tracings (coagelgrams) of the process of coagulation elicited through recalcification of citrated plasma. It was found that *the first formation of fibrin occurs at point F of the coagelgram. Between points F and C continuous formation of fibrin takes place. At point R is noted the first onset of retraction of the clot with expression of serum.*

The three points of the coagelgram divide it into four parts. These four divisions represent four different phases of the process of coagulation. These have been termed: (1) *the period of dissociation,* (2) *the period of fibrin formation,* (3) *the rest period,* and (4) *the period of clot retraction.*

The coagelgram further indicates a fifth variable; namely, the relative reduction of transmitted light during the process, this relative reduction being equivalent to the quantity of fibrin formed, thus constituting the basis for a quantitative method

Through ultramicroscopic observation and photography of the process of coagulation, a morphologic basis is presented, substantiating the above interpretation.

It was noted that *a change in the size and the outline of platelets with clumping of the platelets preceded the actual formation of fibrin.* Further, *the rapidity with which these morphologic changes occur in the platelets appears related to the velocity of the entire process.* It cannot be stated whether or not the velocity of these changes in the platelets merely coincides with the velocity of the entire reaction or actually governs it.

According to Hammarsten's classical conception, the process of coagulation is divided into two phases. (1) the formation of thrombin, and (2) the transition of fibrinogen into fibrin A modification of this view is presented It is suggested that these two processes, although qualitatively different, as concerns time run parallel from the beginning of the first formation of thrombin until all fibrinogen has been converted The expression may be used that *the two processes, as concerns time, are telescoped one into the other. The period of dissociation,* as indicated by the coagelgram, *would then cover mainly that*

second stage were known to be on essential points based on deficient technique These investigations were undertaken in cooperation with Gathe and Guthe.[2] We had at the time no opportunity to determine the absorption band of fibrin and therefore could not satisfy the requirement of the employment of suitable filters. With the low intensity of illumination as maintained also for these studies and corresponding to 9.5 microamperes, a linearity between the illumination and the current output of the cell, however, was assured

Another difficulty was encountered in deciding upon the measurement of the relative reduction of transmitted light between points F and C.

An exact measurement of the photo-electric values of points F and C as expressed in microamperes was excluded by technical reasons.

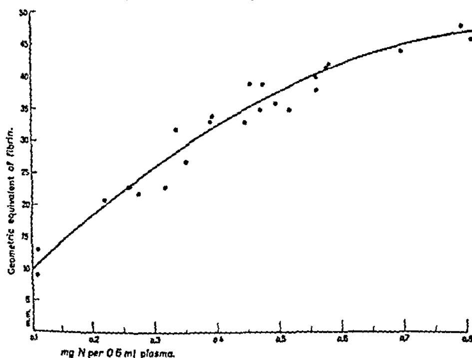


Fig 16.—Curve correlating mg N per 0 6 c.c. citrated plasma with the geometric equivalent of fibrin as read directly off the coagelgrams (in millimeters)

On the basis of the linearity between illumination and current output a geometric equivalent of the reduction of transmitted light between points F and C may be had by measuring in millimeters the vertical distance between these two points of the coagelgram. In Fig 17 this geometric equivalent Y is clearly indicated

Now it is obvious that by employing a standardized technique the maximal value of transmitted light to be found at the very beginning of the coagelgram would remain constant from one observation to the other and correspond to the stated 9 5 microamperes Due to the previously mentioned preliminary

CHAPTER V

THE FIFTH VARIABLE OF THE COAGELGRAM

Outline of a Photo-Electric Approach to Quantitative Determination of Fibrin

According to the preceding interpretation the coagelgram indicates the division of the process of blood coagulation into four separate stages. In addition the coagelgram presents a fifth variable. Its physical exponent is represented by the relative reduction of transmitted light during the second stage of the process. The characteristic of this second stage is the continuous formation of fibrin and, according to the preceding chapter, the sole process responsible for the reduction of transmitted light during this second stage. In order to verify this contention it was suggested to attempt a direct correlation of this relative reduction of transmitted light with the quantity of fibrin as determined by one of the regularly employed quantitative procedures.

In its simplified form this latter problem is similar to a variety of quantitative procedures based on photo-electric technique. Their success is largely dependent upon a satisfaction of certain fundamental physical requirements These warrant brief consideration

According to the absorption laws of Lambert and Beer the concentration of a substance under investigation is proportional to the negative logarithm of the unabsorbed light The provision is that the investigative technique includes the use of spectral filters transmitting light corresponding to the absorption band characteristic of the substance under investigation.

The unreserved application of Lambert and Beer's law to photo-electric investigation can rarely be expected for the following reasons as quoted from Sanford, Sheard, and Osterberg [1]

"1. Difficulty in obtaining filters which are feasible for ordinary technical routine and which have transmission bands corresponding exactly to the characteristic absorption band or zone selected. The curve of transmission should be the mirror image of the curve of absorption. 2 Variability in or change of shape of absorption band and therefore the values of the absorption of light for the various wave lengths involved, with degree of concentrations of the material in solution Strictly speaking, the law that $C = -k \log I$ holds only under the condition that the measurements of unabsorbed light are made at specified wave-lengths such as that corresponding to the maximal absorption of light. 3. Lack of linearity or proportionality in the relationship between intensity of illumination I and the current A developed."

On this basis the preliminary investigations aiming at a correlation of the quantity of fibrin and the relative reduction of transmitted light during the

This curve may be expressed by the power series:

$$Y = a + bX + cX^2 + \text{--------------}$$

Y denotes the geometric equivalent of fibrin and X denotes milligrams of nitrogen per identical volume of citrated plasma.

A closer consideration reveals that the first three terms will give sufficient approximation. A numerical valuation of the constants will then give:

$$Y = 105.6\ X - 61.0\ X^2.$$

This equation is not applicable to values of X exceeding 0 8 and does not permit extrapolation. These reservations are overcome by the use of an exponential curve on the basis of the following equation:

$$Y = 62\,5\ (1 - e^{-1\,77X}).$$

These findings represent a substantiation of the interpretation of the coagelgram as concerns the meaning of the second stage of blood coagulation. They further indicate the possibility of quantitative estimation of fibrin on the basis of photo-electric tracings of recalcified citrated plasma.

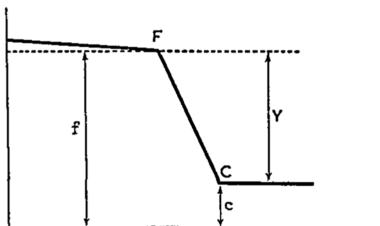


Fig 17 —Diagrammatic sketch of a simplified coagelgram *F* indicates first formation of fibrin, *C*, completion of fibrin formation, *Y*, the geometric equivalent of fibrin, to be measured *in millimeters*, *f* and *c*, *deflection of ammeter mirror in points F and C, respectively*

With the latter possibility in mind I sought the cooperation of Dr. Helmer Dahl, chief of the Geophysics Institute of Bergen.

Our primary intention was through mathematical and physical considerations of the present and related problems to obtain more detailed information regarding essential requirements for successful quantitative procedures based on the photo-electric principle A brief outline of these considerations is included here as they may be of some value to others confronted with similar problems

The following discussion is simplified by viewing it in direct relation to the quantitative estimation of fibrin, although it holds true for any similar problem of identical nature.

changes of platelets during the first stage of the coagulation of recalcified plasma a small reduction of transmitted light would occur during this first stage. As indicated, the degree of this reduction would vary somewhat with the velocity of the entire process. Consequently the value of transmitted light corresponding to point *F* would vary slightly from one observation to the other. In relation to the much more extensive reduction of light between *F* and *C* these smaller variations of *F* introduce no essential error. With this reservation the equivalent *Y* as measured in the indicated manner has been retained.

On the basis of these known deficiencies a series of correlated investigations were performed.

Employing the standardized technique (page 123) photo-electric tracings of the coagulation of recalcified citrated plasma were obtained in twenty-four specimens from twenty individuals Simultaneously the content of fibrinogen was determined in each identically treated specimen according to the micromethod of Kjeldahl. A double set of observations was obtained. In order to reduce the inaccuracy of the Kjeldahl procedure invariably associated with too minute volumes of plasma, 3 c.c. of citrated plasma was coagulated with 1 c.c. of the solution of calcium chloride. The final result was expressed as per 0.6 c.c. of plasma to correspond to the volume of plasma used in obtaining the photo-electric tracings. In Table IV is listed the correlated value of the geometric equivalent and the milligrams per 0.6 c.c. of citrated plasma. The curve fitted on the basis of these data appears from Fig. 16.

TABLE IV

CORRELATED VALUES OF QUANTITIES OF FIBRIN AND THE FIFTH VARIABLE OF THE COAGELOGRAM

OBSERVATION NO	GEOMETRIC EQUIVALENT OF FIBRIN (MM.)	MG. N PER 0.6 C.C. OF CITRATED PLASMA
1	46 0	0.800
2	38 0	0.536
3	20.7	0.220
4	35 0	0.514
5	32 5	0.396
6	39 0	0.472
7	41 3	0 572
8	23.0	0 260
9	23 0	0.258
10	22 0	0 274
11	36 0	0.492
12	42 0	0 576
13	34 5	0 468
14	26 5	0.350
15	32 7	0.444
16	34 0	0.392
17	44 5	0 690
18	39.0	0 452
19	32 0	0.335
20	23 0	0.316
21	48.0	0.784
22	13 0	0.111
23	9 0	0.104
24	40.0	0.556

Inserting the latter in (5), Y may be expressed thus:

$$Y = g\,I\,e^{-\frac{c_t}{k}}\,(1 - e^{-\frac{1}{k}X}) \qquad (7).$$

Combining (2) and (7):

$$Y = g.I_t\,(1 - e^{-\frac{1}{k}X}) \qquad (8).$$

In this latter equation k is the constant of Lambert and Beer's law and g *the constant of the photo-electric sensitivity. These constants are independent of the factors Y and X. These two factors thereby obtain an unambigous* correlation.

It will be noted that this equation (8) is identical to the exponential equation stated above *as satisfactorily approaching the empirically obtained curve correlating the two values Y and X. The deviations from the theoretic curve of the actually observed values of Y and X indicate the extent to which the* employed photo-electric procedure has failed *to satisfy* the physical requirements considered to be ideal and the prerequisite for the unreserved application of the absorption law of Lambert and Beer.

On the basis of the results of our observations and the present discussion *it appears that the photo-electric principle as here employed may be advantageously employed in the quantitative estimation of blood fibrin.* Because of unsettled conditions it has been impossible to follow up this lead with a series of control observations which might have formed the basis of a routine technique for photo-electric determination of blood fibrin

References

1. *Sanford, A. H., Sheard, Charles, and Osterberg, A. E.* The Photelometer and Its Use in the Clinical Laboratory, Am. J. Clin. Path. 3: 405-420, 1933; Coll. Papers, Mayo Clin. & Mayo Foundation 25: 1060-1083, 1933.

2. *Nygaard, K. K., Gathe, R., and Guthe, Th.:* Quantitative Determination of Blood Fibrin by the Photoelectric Method, Skandinav. Arch. f. Physiol., in press

Referring to our correlated determinations above, the results were obtained under technical conditions admittedly not satisfying the theoretic requirements. The question remained how these results would compare to the ones to be expected if these requirements were satisfactorily met; that is, when working with monochromatic light and ideal filter technique. Under the latter ideal conditions the law of Lambert and Beer is applicable without reservation and is the provision for the following considerations:

Denote by I the light entering the solution under investigation; I_f, the light falling onto the photo-electric cell after its passage through the solution; c_f, the concentration of the substance under investigation at point F; and c_c, its concentration at point C.

According to the law of Lambert and Beer:

$$c_f = - k \ln \frac{I_f}{I} \qquad (1).$$

or

$$I_f = I e^{-\frac{c_f}{k}} \qquad (2).$$

where k is a constant.

In the same way the light I_c falling upon the cell at point C might be expressed thus:

$$I_c = I e^{-\frac{c_c}{k}} \qquad (3).$$

Further, considering linearity between light intensity and photo-electric current and designating the deflection of the ammeter mirror in point F as f and in point C as c (Fig. 17), we may write:

$$\begin{aligned} f &= g.I_f \\ c &= g\, I_c \end{aligned} \qquad (4).$$

The difference between f and c $(f-c)$ constitutes the deflection of the ammeter mirror during the process of fibrin formation and is identical to the vertical lineary distance between points F and C of the coagelgram. This geometric equivalent Y can be expressed:

$$Y = f - c = g I \left(e^{-\frac{c_f}{k}} - e^{-\frac{c_c}{k}}\right)$$

or

$$Y = g I.e^{-\frac{c_f}{k}} \left(1 - e^{-\frac{c_c - c_f}{k}}\right) \qquad (5).$$

Terming the variation in the concentration of the substance (fibrin) under investigation X, we have

$$X = c_c - c_f \qquad (6).$$

In the description of the technique of various coagulation tests it is indicated that the observations are performed at room temperature. This introduces an uncontrollable factor. It is well to remember the great variations in temperature in various geographic regions as well as the great seasonal variations noted in the same region. These variations were pointed out in 1908 by Addis,[1] who concluded that "where no attention is paid to differences of temperature the method is practically worthless." On this ground Minot[2]

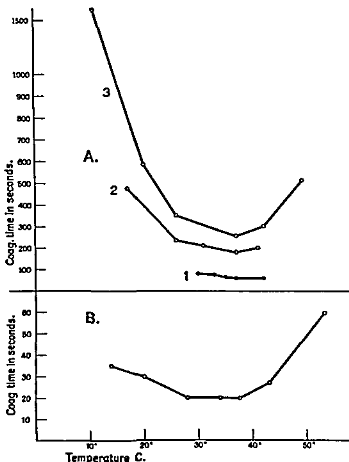


Fig 18 —Variations in velocity of reaction with varying temperature. *A*, Three different specimens of recalcified citrated plasma, *B*, one specimen of citrated plasma coagulated by Mellanby-Bleibtreu thrombin solution.

criticized Howell's original technique which included observations at room temperature Gram[3] took full cognizance of this fact by performing his observations at temperatures of 35° C, obtained by the use of an unsilvered half-liter dewar vacuum flask with holes for the miniature test tubes

Aside from points of practical consequence, it is only reasonable to assume nperature variations may give rise to theoretic considera-

PART II

CHAPTER VI

EXPERIMENTAL INVESTIGATIONS ON THE COAGULABILITY OF BLOOD PLASMA

The Effect of Variations in the Temperature

It is well known that the coagulation time changes with variations in the temperature of the specimen of blood. The more accurate methods have taken this into account by standardizing the technique at a fixed temperature.

In order to evaluate the importance of the effect of temperature variations with the present technique, the following investigations have been undertaken

From a series of patients, citrated plasma has been prepared by withdrawing fixed volumes of whole blood into fixed volumes and quantities of sodium citrate solution After sedimentation of the erythrocytes the plasma has been pipetted off from the individual specimen, deposited in a test tube where the plasma is mixed gently, following which 0 6 c.c. of plasma is transferred to a series of absorption cells. Each cell is inserted into the water bath *for 15 minutes to obtain the temperature at which the observation is to be performed.* The solution of a fixed concentration of calcium chloride is likewise kept in the water bath for the same length of time After ascertaining that the plasma and the solution of calcium chloride have the same temperature as that recorded by the thermometer of the water bath, the process of coagulation is induced by adding 0.2 c.c. of the calcium chloride solution to the plasma. The coagelgrams of the same specimen obtained at the various temperatures have been read according to the technique previously described. The results of three such serial observations are recorded in Fig. 18*A*.

In a second series of investigations the procedure has been varied slightly by inducing the coagulation of citrated plasma with a Mellanby-Bleibtreu thrombin solution instead of using calcium chloride. Otherwise, the technique has been identical. The result of such an observation is recorded in Fig 18*B*.

The graphic illustration of the result of these studies is self-explanatory. It clearly indicates that *the maximal velocity of the process is found at about 37° C., and further, that the velocity is reduced at lower as well as higher temperatures. The same is found to be true with coagulation of citrated plasma by a thrombin solution.*

These findings indicate that it is necessary to keep the water bath at a constant and fixed temperature to assure comparable observations. It is important to keep the specimen to be tested and the recalcifying solution in the water bath a sufficient length of time before the test is made to allow them to attain the temperature of the water bath

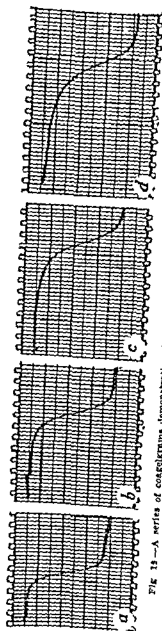


Fig 13 —A series of coagelgrams demonstrating the effect of centrifugation of the plasma (see Table V).

tions This may turn out to be a problem of no small magnitude, based as it must be on concepts of still hypothetic value concerning the nature of the process of coagulation, whether this be chemical, enzymatic, or some other type of reaction. For this reason, I have included observations on the effect of temperature variations on the coagulation of citrated plasma by thrombin. The similarity of the velocity changes in relation to the various temperatures at which this reaction is observed may indicate that, at least to a certain extent, the variations in temperature may exert an influence through the thrombin activity. This possibility may lead directly to the question of the temperature coefficient of various enzymes.

Effect of Centrifugation of the Plasma

Mosen[4] studied the variation in the number of platelets after centrifugation of oxalated plasma. He described the various layers formed by the cellular elements following centrifugation of the plasma, the lowest being a heavy layer of red cells, then a thick grayish red layer of leucocytes, with a fine veil-like white layer of platelets on top. By recalcification of specimens before and after centrifugation, he found relative prolongation occurred after centrifugation. This observation has been substantiated repeatedly by various investigators Lee and Vincent[5] concluded that the coagulation time of recalcified oxalated plasma depends on the number of blood platelets in it.

For investigation of this point, the following procedure has been used: Blood is withdrawn in the usual manner, using 9 volumes of blood and 1 volume of a solution of sodium citrate. After gentle and careful mixing the specimen is deposited in a series of test tubes in equal volumes. All tubes have been left in the refrigerator for 4 hours to permit sedimentation of the erythrocytes All test tubes except the first are then centrifuged separately; the duration and the speed of centrifugation are recorded in each case. The supernatant plasma in each tube is partly pipetted off and deposited in separate individual test tubes, from which a constant volume of plasma is transferred to the absorption cells. In each case, before recalcification and recording of the process of coagulation, a drop is removed from the plasma of the absorption cell, transferred to the counting chamber, and the platelets are counted according to a previously published method[6] In plasma of high platelet counts it is deemed advisable to dilute the citrated plasma with a solution of sodium citrate In Table V is recorded the effect of centrifugation on the number of platelets in the plasma. Fig. 19 illustrates the simultaneously recorded coagelgrams of the identical series.

TABLE V

THE EFFECT OF CENTRIFUGATION OF THE PLASMA ON THE NUMBER OF PLATELETS

SAMPLE	REVOLUTIONS PER MINUTE	DURATION OF CENTRIFUGATION	PLATELETS PER CU MM.
(a)	–	No centrifugation	395,000
(b)	1,000	10 minutes	195,000
(c)	2,000	10 minutes	51,000
(d)	3,000	30 minutes	3,500

So far as the coagulation of the plasma is concerned, I have not been able to find that this process in any way simulates a photochemical process. By the use of selective filters of different spectral ranges no definite changes have been found to be produced in the velocity of the process of blood coagulation. In the present problem the constancy of illumination serves only the latter of the two purposes mentioned above. This definitely simplifies the technique. A few important points, however, are to be considered.

The translucency of various plasmas may vary greatly because of the relative number of thrombocytes, the presence or absence of transitory lipemia, or the inherent color of the serum. Due to variation in translucency of the different specimens, it is obvious that no constancy of photo-electric registration is obtained by maintaining a constant source of light. Such a constancy would be only illusory. The variation of translucency has to be compensated for. *This may be done on the basis of the quantity of light reaching the photo-electric cell after the light has passed through the specimen.* This value is easily standardized. During this procedure the photo-electric cell with its amperemeter registers the photo-electric equivalent of the intensity of light to be standardized By the use of the coarse and the fine rheostatic arrangement controlling the source of light, the illumination reaching the photo-electric cell may be brought to the constant, arbitrarily chosen value When this value is reached, it is of the greatest importance that it be kept constant.

Observations have been undertaken in order to decide whether this standardization is to be made before or after recalcification of the plasma As a criterion of the results I have used the geometric equivalent of the quantity of fibrin, as dealt with in a previous chapter More exact results are obtained by performing the standardization on the basis of illumination of the photo-electric cell *after* recalcification of the specimen and not before It is further found that this is an absolute necessity when using oxalated plasma instead of citrated plasma for research purposes Calcium oxalate crystals will be precipitated by recalcification and will cause a varying degree of turbidity, according to the concentration of the recalcifying solution By standardizing the illumination after recalcification of such specimens, one may compensate for this interfering factor. This may be illustrated by a simple observation In Fig 20 is presented a series of coagelgrams of a specimen of oxalated plasma recalcified with increasing concentrations of a solution of calcium chloride The standardization of illumination in this series is performed *before* recalcification of the specimen Because of the increasing precipitation of calcium oxalate crystals the geometric equivalent of milligrams of fibrin is decreasing, with concentrations of calcium chloride increasing, in spite of the fact that the fibrin content is identical in all of the specimens

The next figure (Fig 21) presents a series of observations in which the standardization is made *after* recalcification of the specimens Otherwise the experimental conditions are the same in the two series of observations In this series the geometric equivalent of fibrin in all specimens is identical This indicates that the standardization of illumination *after* recalcification of the speci-

The results of a series of similar observations all point in the same direction. The number of platelets is reduced by centrifugation of the specimen, apparently more by the speed than by the duration of centrifugation. However, after centrifugation of 1 hour at high speed, one will frequently find that the plasma is not completely free of platelets. The coagulation time of recalcified oxalated or citrated plasma after centrifugation is definitely prolonged as compared to that of a noncentrifuged specimen. One cannot find more than a very rough proportionality between the number of platelets and the coagulation time, although such a definite relation is maintained by Lee and Vincent. These investigators have found that a saline or a watery extract of platelets was as effective as the platelets themselves. Because of this, I feel rather reluctant to accept the strict proportionality between the numbers of platelets and the coagulation time, as it is quite possible that a certain percentage of the platelets in the plasma may have lost their discreteness by the centrifugation or for other reasons, although their coagulant effect may be retained Additional factors of uncertainty may be represented by the unknown coagulant effect of various types of platelets (large, middle-sized, small, young and older types). Mention may be made at this time of a commonly made observation that specimens with an originally low platelet count exhibit a more marked prolongation after centrifugation than specimens of high or higher platelet number. Roughly, it appears as if there exists a certain level of platelet number below which a reduction of coagulant is appreciably present.

The practical conclusion from these investigations is that, in order to obtain comparable results, centrifugation of the specimens ought to be avoided

Variations in Illumination

One of the crucial points of the successful applicability of the photo-electric technique to qualitative or quantitative determinations of any kind is the question of the constancy of the source of light. Even under the most ideal conditions this problem needs further qualification. One has to discriminate between the constancy of the source of light and the more relative constancy of transillumination. A couple of examples may clarify this point. The photo-electric method for quantitative determination of ascorbic acid as described by Guthe and me[7] illustrates the importance of the constancy of the source of light. The principle of this method is based on the decoloration of a known concentration of methylene blue in the presence of ascorbic acid under the influence of light of a certain wave length The interrelation of these three factors is quantitative. To obtain comparable quantitative results the standardization of the method must be undertaken on the basis of a constant quantity of light of specified nature, this light being a quantitative participant in this photo-electric reaction. In this reaction the light consequently serves two purposes. first, furnishing a constant quantity of photochemical energy, and second, furnishing a constant illumination whose photo-electric equivalent may serve as the base line for comparison with subsequent photo-electric changes following reduction of the methylene blue solution.

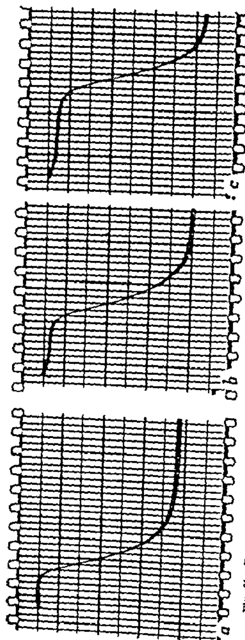


Fig 21.—Demonstration of the compensation of interference from increasing calcium-oxalate precipitation through standardization of illumination *after* recalcification of the plasma. Same specimen and setup as in preceding figure. $CaCl_2$ in *a*, 0 5, in *b*, 1 0, and in *c*, 1 5 per cent, respectively *Note*. The velocity of reaction is not expected to be identical in the three recordings.

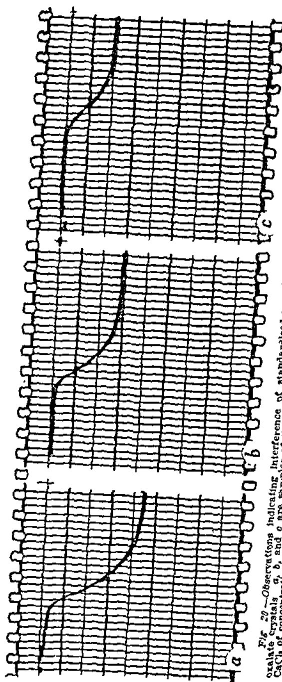


Fig 20—*Observations* indicating interference of standardized recording through increasing precipitation of calcium-oxalate crystals *a*, *b*, and *c* are samples of the same oxalated plasma. Recalcification performed with a solution of $CaCl_2$ of concentrations, *a*, 0 5, *b*, 1 0, and *c*, 1 5 per cent, respectively. In *each specimen illumination* is identical *before* recalcification NOTE The velocity of the reaction is not expected to be identical in the three recordings.

men is the correct procedure It further illustrates and emphasizes a technical point of essential importance for the successful application of the photoelectric principle to the quantitative determination of blood fibrin.

In this chapter I further want to present studies related to changes in the coagelgrams following variation in the illumination. In these experiments the standardization of illumination has been performed after recalcification of citrated plasma The variation of illumination in these series consequently is not relative. In Fig. 22 is presented a series of coagelgrams of a specimen of citrated plasma, the various samples of the specimen treated in all respects identically. The illumination of the photo-electric cell after recalcification of the specimen was decreased from 12.1 to 4.1 microamperes

From a series of similar observations a few points of interest may be noted: By variation of illumination (a) the coagelgrams retain their original shape, (b) the duration of the first and the second periods of the process does not undergo any variation, and (c) the point of maximal velocity of fibrin formation retains its position in relation to the zero point of the reaction, as far as time is concerned. In other words, *the velocity of the reaction undergoes no change by variation of illumination.* From a practical point of view this is of importance In investigations of the coagulability of the blood where photoelectric technique is employed, *standardization of illumination is not an absolute requisite in case the technical device is to be used for investigations of velocity only* Even so, however, such a standardization is desirable for the following reason: With low illumination and great turbidity of the specimen one may obtain a flattened coagelgram which by its form would indicate an impairment of coagulability, while in reality it may take the form of a perfectly normal coagelgram under standardized, suitable physical conditions, *signifying normal* coagulability.

The most conspicuous variation in the appearance of the coagelgrams of Fig 22 is represented by the geometric equivalent of the quantity of fibrin decreasing with the decreasing value of illumination, while in reality the fibrin content is identical in all. This variation is readily understood on the basis of the laws of Lambert and Beer Aside from the theoretic interest, these variations furnish a point of practical consequence for the present investigations.

The photelgraph is constructed for the use of light-sensitive paper with a width of 6 cm It is naturally of practical convenience to arrange for an investigative setup which permits the recording of unbroken coagelgrams in as many cases as possible even with a fairly high fibrin content in a group of the material It is equally desirable at the same time to arrange it so that recordings of the process in normal persons or in patients with low quantities of fibrin still permit a convenient reading of the various phases of the process, particularly the point of maximal velocity of the fibrin formation Considering then the width of the film, the quantity of plasma to be employed (0 6 c c.), the dimensions of the absorption cells, and the distribution of the quantity of fibrin under varying clinical conditions, I have standardized the present investigative technique on an arbitrarily chosen, fixed value of 9.5 microamperes

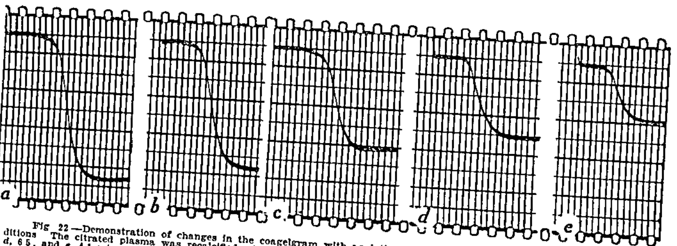


Fig 22 —Demonstration of changes in the coagelgram with variation of illumination under otherwise identical conditions The citrated plasma was recalcified with $CaCl_2$, 1 0 per cent. The illumination equal to · *a*, 12 1; *b*, 10 2; *c*, 8.2; *d*, 6 5, and *e*, 4 1 microamperes, respectively. NOTE The velocity of reaction was not expected to change from one sample to the other in this series

By increasing the concentration of calcium above the required minimal concentration, the coagulation time is shortened gradually until it reaches a relative minimum, a point of practical consequence as considered by several workers (Howell,[18] Gram,[3] Smith,[14] Nygaard,[17] Von Zarday,[16] and others). More exact investigations regarding this point have been undertaken by Ransmeier and McLean.[13] They computed the minimal coagulation time of diluted citrated plasma as occurring at a concentration of calcium ions at or above 1.25 mM. per liter at a pH of 7.0 to 8.0 (average minimal coagulation time 10 2 and 5.7 minutes for human and dog plasma, respectively). These workers further presented an empirical formula for the estimation of the coagulation time (t) of a given plasma:

$$t = \frac{K}{Ca^{++} - m} + n.$$

where K is a constant, Ca^{++} is calcium ion concentration in mM. per liter, n is a constant expressing coagulation time at infinite Ca^{++} concentration; and m is a constant expressing Ca^{++} concentration for clotting at infinite time. The practical value of this formula is greatly limited because the constants vary from sample to sample.

By increasing the calcium concentration still more, the calcium exerts an anticoagulant influence. This fact was first noted by Horne,[19] who found that barium and strontium, however, exhibited greater anticoagulant action in higher concentration as compared to the calcium. By dilution with distilled water or by the addition of definite quantities of potassium oxalate the coagulant effect of these salts was restored. These investigations by Horne probably represent the very first clear intimation of the quantitative importance of calcium for the process of blood coagulation. His findings regarding the latter point were verified by Sabbatini,[21] who determined the anticoagulant action of calcium as occurring at a concentration of 18 Gm of calcium chloride per liter (162 mM.) Horne's investigations regarding the relative effect of calcium strontium, and barium on coagulation have been carried on by Buglia[23] (1906) and Lehmann[22] (1922). The latter, in variance with the results of Horne, found that the anticoagulant effect of barium, calcium, and strontium increased in the order mentioned

By proceeding to a consideration of the quantitative relationship of the most commonly used anticoagulants and their relation to calcium, it goes without saying that calcium is considered a coagulant although in higher concentrations it may act as an anticoagulant.

In a previous chapter the necessity of adding the decalcifying solution in excess of the equimolecular calcium to prevent coagulation is considered and the controversy arising from this finding.

Sabbatini[21] is credited with being the first to determine this relationship quantitatively He found that a minimum of three molecules of the recrystallized citrate for each atom of available calcium was necessary for the prevention of coagulation of dog's blood in vitro

of unabsorbed light *after* recalcification of the specimen. On this basis only a relatively few specimens of very high quantities of fibrin produce relative reductions of transmitted light during the second stage necessitating the recording of the process in broken coagelgrams (see Fig. 10).

The Interrelation of the Anticoagulant and Coagulant Solutions

In a previous section are reviewed some of the more basic works regarding the importance of calcium for the coagulation of blood, also the mechanism by which oxalate and citrate solutions act as anticoagulants.

In this section the quantitative relationship of these anticoagulant and coagulant solutions, and their effect on the velocity of the process of coagulation, will be considered.

After Arthus and Pagès[8] had demonstrated the necessity of active calcium ions for the clotting of blood in vitro, considerable work had been undertaken to determine the actual minimal requirement of calcium.

An early attempt in this direction is represented by the works of Stassano and Daumas.[9] For their investigations they used salted plasma brought to coagulation by dilution with water or with a solution of calcium chloride. They found that an addition of calcium below 13 mg per liter of plasma did not produce coagulation.

Of recent years Nordbö's[10] research on this point is important. By carefully controlled experiments, he investigated the minimal concentration of active calcium ions necessary to produce coagulation of the plasma within 24 hours at 25° C. Nordbö used plasma from animals for his studies. In plasma with strong electrolytes of a concentration of 0 16 M. per kilogram of water, the minimal concentration of ionized calcium in plasma equalled 0.15 to 0.18 mM. per kilogram. According to Nordbö's investigations this concentration equalled a calcium ion activity in plasma of 0 04 to 0.05 mM. per kilogram of water. By increasing the concentration of the electrolytes in the plasma, this minimal concentration or even higher concentrations may not effect coagulation as the ion activity of calcium is thereby reduced Similar findings were later presented by Lebel and his associates [11]

Ransmeier and McLean,[12] apparently without cognizance of the works of Nordbö and on the basis of the investigations of Hastings, McLean and associates, computed the minimal requirements of calcium ions for coagulation of diluted citrated plasma at an average of 0.35 and 0.24 mM per liter for plasma from human beings and dogs, respectively. These investigations were performed at a pH of the plasma of 7 0 to 8 0 A change in the pH below or above these values necessitated an increase in the minimal concentration to effect coagulation.

It may be mentioned here that calcium can be substituted by the other alkaline of earths, a fact partly observed by Arthus and Pagès and later amplified by studies of Mellanby[13] and Heard.[14] Heard found that none of the metallic cations produced coagulation as they resulted in the precipitation of emulsoid protein solutions

the recalcifying solution while the anticoagulant solution remained constant (9 volumes of blood to 1 volume of 3.8 per cent trisodium citrate).

From Fig. 23 it will be apparent that the coagulation time of the recalcified citrated plasma is fairly constant between concentrations of calcium of 0 7 and 1.3 per cent calcium chloride.

In the present experiment the recalcifying solution exerts *a maximal coagulant effect* between these concentrations With increasing quantities of calcium there is noted a gradual prolongation of the coagulation time. With quantities below those exerting a maximal coagulant effect a gradual prolongation of the coagulation time is apparent at first, which becomes a sudden and very marked prolongation. It is to be noted that this sudden and marked prolongation is effected by comparatively minute reductions in the quantities of calcium. The curve is here approaching *the minimal value for calcium ions* necessary for the coagulation of this particular specimen. Excepting that part

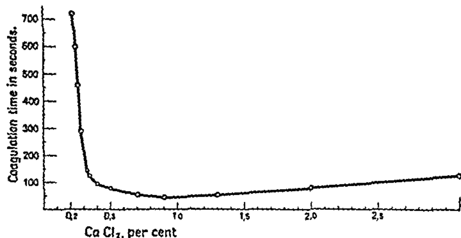
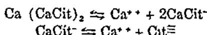


Fig. 23 —Curve graphically representing the variations in the coagulation time of recalcified citrated plasma by varying concentrations of $CaCl_2$. The anticoagulant solution was 3 8 per cent sodium citrate (9 parts of blood to 1 part of sodium citrate)

of the tracing indicating slight prolongation with higher quantities of calcium, the rest of the curve appears practically identical to the one illustrated in the work of Rausmeier and McLean and by them described as having the form of a rectangular hyperbola By using oxalate as an anticoagulant solution an identical type of curve is encountered.

Using a procedure in all respects identical to the one just described, identical types of serial observations have been undertaken in a good many normal persons and in patients with various diseases The results of some of these observations are illustrated in Fig 24 Each curve represents serial observations of the specimen from different individuals. The curve at the bottom of Fig 24 (Curve 1) is a reproduction of the one of Fig 23 Curves 4, 5, and 6 are obtained from specimens taken from two patients with obstructive jaundice and one from a hemophiliac, respectively These three patients had clinical evidence of hemorrhage

Mention ought to be made of a question which at one time occupied the interest of the workers concerned with the quantitative estimation of blood calcium, a point, however, which appears of some consequence in the present connection. This concerns the combination of blood calcium with blood citrates. On the basis of their investigations, Nordbö and Scherstén[24] concluded that, normally at least, such a combination did not amount to more than about 1 mg. per cent of calcium. Notice ought to be taken of the findings of Sjöström[25] that in certain clinical conditions the blood citrates may be materially increased However, this may be of little consequence in the present connection, according to the investigations of Hastings and his associates.[26] Their works indicate that calcium, magnesium, and strontium citrates do not exist in nonionized form but from a practical point of view may be considered existing in ionized form so far as the diffusible calcium is concerned. According to Hastings and his associates this dissociation may be considered in the following equations representing the primary and the secondary dissociation of calcium citrate:



$$Ca\ (CaCit)_2 \leftrightharpoons Ca^{++} + 2CaCit^-$$
$$CaCit^- \leftrightharpoons Ca^{++} + Cit^{\equiv}$$

Mention should also be made of the investigations of Peretti[27] concerning the des-ionizing influence by citrates of calcium salts under varying pH (from 1.4 to 7.5). At a pH above 6.0 a relative minimum of citrate was required for the des-ionization of calcium, the quantity rapidly increasing to a maximum about the pH of 3 5 from which point there was a gradual decline to a pH between 2 6 and 1.4, however not reaching the minimal value at the pH above 6 0.

Its Effect on the Velocity of Coagulation.—In investigating some of the points mentioned in the introduction to this chapter and which are of particular consequence for the present work, I have proceeded as follows:

Where nothing otherwise is stated the specimens of blood are obtained in the following manner 9 parts of blood are withdrawn into a graduated syringe containing 1 part of trisodium citrate ($2[C_3H_4OH(COONa)_3]11H_2O$). After sedimentation of the erythrocytes the supernatant plasma is pipetted off. From this plasma 0.6 c c is transferred to the absorption cell for each observation. To this is added 0 2 c c. of a solution of calcium chloride ($CaCl_2 + 6H_2O$), the point of addition serving as the zero point of the process of coagulation. The observations are all performed at the constant temperature of 37 5° C. The resulting coagelgrams are read according to the technique described in a previous chapter. The results of these investigations are presented in the form of graphs in order to avoid reproduction of the original coagelgrams, the appearance of which has to be translated into time in order to concentrate the results of the investigations When determinations of the hematocrit have been undertaken, they have been done by centrifugation of the original blood specimen for 20 minutes at 3,000 revolutions per minute after removal of the necessary plasma for the coagulation tests.

Varying the Concentration of the Recalcifying Solution—The results as graphically presented in Fig. 23 are obtained by varying the concentration of

whether the comparison is undertaken at one or the other of the quantities of calcium affecting coagulation.

One exception to the statements given above ought to be made. As will naturally follow from the subsequent exposition, the expression "standardized conditions" as used above will also have to include a consideration of the hematocrit value of the specimen under investigation. Specimens 1, 2, 3, and 6 of Fig. 24 are all of normal hematocrit value. Specimens 4 and 5 are both of reduced hematocrit value. As to be shown later, this necessitates a qualification of the interpretation of the relative coagulability of these two specimens This does not change the essentials of the statements given above.

From Fig. 24 it is further to be noted that the anticoagulant action of calcium in higher concentrations is increasing with reduced relative coagulability of the specimen.

It is equally evident that in the present system the minimal quantity of calcium necessary for the coagulation of citrated blood plasma is increasing with reducing coagulability of the specimen.

A logical consequence of the latter two points is that the range of maximal coagulant effect of calcium is getting more and more limited with reducing coagulability. This point is brought out fairly well by Fig. 24 This process of limitation appears to be centered around a point which may be considered the very maximal point of coagulant effect of calcium (in the present system approximately 1 0 to 1.4 per cent calcium)

Before undertaking a comparison of the results of these investigations with previous researches, it seems convenient to touch on a few points of consequence for subsequent computation. The previously reviewed results of the quantitative addition of calcium are generally expressed per liter of genuine plasma or per kilogram of water of the plasma With the present technique of obtaining our blood samples, it is evident that the hematocrit value has to be taken into account.

In Table VI is computed the actual volume of genuine plasma per 0.6 c c of citrated plasma in relation to the value of the hematocrit of the specimen.

TABLE VI

COMPUTATION OF THE ACTUAL VOLUME OF GENUINE PLASMA AND ANTICOAGULANT SOLUTION IN 0 6 C C CITRATED PLASMA IN RELATION TO VARYING HEMATOCRIT VALUES

(ORIGINAL SPECIMEN 9 C.C. WHOLE BLOOD TO 1 C.C. CITRATE SOLUTION)

HEMATOCRIT PER CENT	VOLUME GENUINE PLASMA (C C.)	ACTUAL VOLUME ANTICOAGULANT SOLUTION (C.C.)
10	0.534	0.066
20	0.527	0 073
30	0.518	0 082
40	0.506	0 094
50	0 491	0.109
60	0.470	0.130
70	0.438	0.162
80	0.386	0 214
90	0.284	0.316

In Fig. 24 several points of interest are noted: The curves here presented run fairly parallel as a group. Under the standardized conditions stated this is taken to indicate that the relative difference in the coagulation time of the different specimens at a given quantity of coagulant solution is relatively maintained throughout this serial observation. In other words this may be expressed that *under standardized conditions the relative difference between the coagulation time of two specimens at a given quantity of coagulant solution is an expression of the variation in a hematologic factor inherent in the specimen, of qualitative or quantitative nature. Briefly, this variation is an expression of the relative coagulability of the specimen of blood under investigation.*

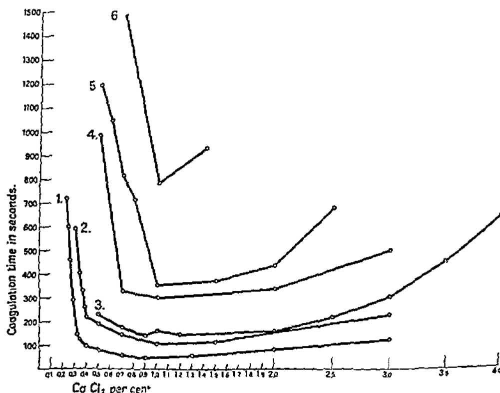


Fig. 24.—Curves graphically representing the variation in the coagulation time of recalci- [illegible] concentrations of $CaCl_2$. The blood specimens were from vari- [illegible] hematocrit [illegible] hematocrit [illegible] coagulant [illegible] 1 part of [illegible]

It is apparent that this finding is of practical consequence Employing a standardized technique, the relative coagulability of a specimen of blood may be determined at an arbitrarily chosen, fixed quantity of the recalcifying solution. This point is to be closer qualified in the subsequent exposition In referring again to Fig. 24 it may be obvious that the specimen of Curve 3 possesses an impaired ability to clot as compared to Specimen 1, while it exhibits higher ability to clot in comparison to Specimen 6, this being the case

into *increasing volumes* of anticoagulant solution of identical concentration. The result of such an experiment is recorded in Fig 25.

It will be seen that the coagulation time of Specimen A_1 for identical quantities of calcium is reduced in relation to Specimen A_2. This is obviously not the result *of an inherent hematologic factor varying in the two specimens*, as they are both taken from the same individual at the same time. The difference in coagulation time is due to the difference in the actual quantity of anticoagulant solution present in the two specimens.

Fig. 25 illustrates several points of interest which have been found to hold true in repeated observations of this type; namely, (1) the specimen of the higher quantity anticoagulant material requires a higher minimal concentration of calcium in order to produce clotting; (2) it is less sensitive to small variations of calcium at the range of the minimal quantity of the latter; and

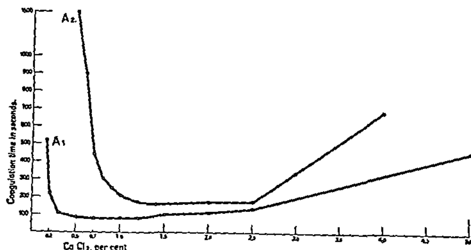


Fig 25—Curves [illegible] plasma by varying concentrations of a [illegible] Specimen A_1 19 c.c [illegible] 15 c.c. whole blood [illegible]

(3) the range of the maximal coagulant effect of calcium is reduced Most important is the finding that (4) the specimen of the higher quantity anticoagulant material exhibits a definitely longer minimal coagulation time of the entire series as compared to that of the specimen with the lower quantity anticoagulant material

B When identical volumes of blood from the same patient have been withdrawn into a *constant volume* of anticoagulant solutions of increasing concentration, identical findings are made. An additional point of interest is then easily brought out, as illustrated in Fig. 26, namely, (5) that the point of maximal coagulant effect of calcium with increasing concentrations of anticoagulant solutions is moving toward higher quantities of calcium.

Aside from several points of theoretic interest these investigations have clearly demonstrated a fundamental requirement of practical consequence.

Table VII records the quantity of calcium (in mM.) of the various concentrations of the coagulant solution (0.2 c.c. of $CaCl_2 + 6H_2O$). It further includes the computation of the millimols of calcium per liter of plasma for a few of the series of calcium chloride solutions used in these investigations and in which the value of the hematocrit of the specimens is 40 per cent.

TABLE VII

COMPUTATION OF MM. CALCIUM ADDED TO CITRATED PLASMA
(STANDARDIZED TECHNIQUE 0.6 C C. PLASMA + 0.2 C.C. $CaCl_2 + 6H_2O$)

PERCENTAGE $CaCl_2$	MM. CALCIUM	MM. CALCIUM PER LITER GENUINE PLASMA OF A SPECIMEN OF 40 PER CENT HEMATOCRIT
0.1	0.00083	1.63
0.2	0.00165	3.26
0 3	0 0025	
0.4	0 0033	
0.5	0.0041	8.14
0.6	0.0050	
0.7	0 0058	11.46
0.8	0 0065	
0.9	0.0074	
1.0	0.0083	16.303
1.1	0.0091	
1.2	0 0096	
1.3	0 0107	21.146
1.4	0 0115	
1.5	0.0124	24 51
2.0	0.0165	32 61
2.5	0.0206	
3.0	0 0250	
3.5	0 0288	
4.0	0.0329	
5.0	0 0412	81 42

On the basis of the illustrations of this chapter and the two tables here presented, data are at hand for a comparison of these investigations with previous ones. The reason why satisfactory comparative investigations are not possible seems obvious enough and will be amplified in the comment to this chapter The computations here outlined as well as the idea of retaining in the illustrations of this chapter quantities of calcium as represented by various percentages of calcium chloride may seem to be a primitive procedure as long as we are dealing with more subtle quantities It is, however, for this very reason that I have proceeded thus. first, in order to avoid giving the impression of a false degree of accuracy, and second, to emphasize the importance of the value of the hematocrit of the various specimens in this type of investigation.

Varying the Concentration of the Decalcifying and the Recalcifying Solutions.—Further investigations have aimed at an understanding of the velocity of the process of coagulation in relation to variations in the quantities of anticoagulants used. For these studies two or more blood samples have been taken from a patient at the same time. The further treatment of the specimens has been identical to that described previously

The approach to these investigations has been varied in two ways.

A. Identical volumes of blood from the same patient have been withdrawn

Fig. 26.—Curves representing the variation in the coagulation time of recalcified plasma [illegible]

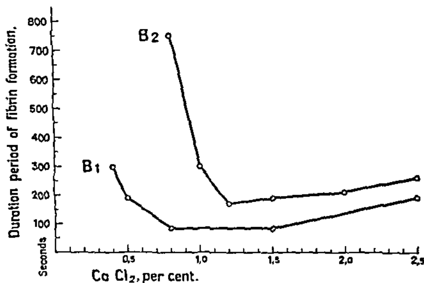


Fig 27.—The duration of the period of fibrin formation in relation to variations in the concentrations of the recalcifying solution. Specimens B_1 and B_2 were taken from the same individual (9 parts of whole blood to 1 part of sodium citrate, of 3 8 and 6 0 per cent, respectively)

Granted that the relative coagulability of citrated plasma may be determined at one arbitrarily chosen, fixed quantity of calcium, the prerequisite is that the specimens to be compared contain a fixed, standardized quantity of citrate per volume unit.

Its Effect Upon the Duration of the Second Stage of the Process.—In connection with the variations in the *coagulation time as recorded above*, the coagelgrams have graphically recorded another variable. This concerns the duration of the period of fibrin formation, that is, the time interval between points F and C in the various coagelgrams.

Whether the coagulation time is defined in one way or the other or determined by this or that method, no conclusions can be drawn a priori as to the duration of that part of the process of coagulation concerned with the formation of fibrin. From a hypothetical point of view it may not appear irrelevant to picture the process of coagulation as one in which the formation of fibrin occurs as an automatic process of constant velocity independent of the duration of the coagulation time The present technique has permitted a separate study of this point.

The observations concerning the duration of the period of fibrin formation have been taken directly from the coagelgrams, the results of which have been recorded in the preceding pages The point of the first formation, F, and the end of fibrin formation, C, have been recorded according to the technique and with the reservations mentioned in a previous chapter

Numerous observations on blood from normal subjects and from patients with various diseases have been unequivocal. For this reason it seems sufficient to demonstrate the variability in the duration of the period of fibrin formation in a simple experiment Two specimens of blood are taken from the same patient at the same time under identical conditions, with the exception of the concentration of the anticoagulant solution. The citrated plasma has been recalcified with varying concentrations of calcium. The variability of the duration of the period of fibrin formation has been recorded in Fig. 27. The specimens here studied are the same as those dealt with in Fig. 26 except that the specimen of the highest concentration of anticoagulant solution is excluded because of the increasing inaccuracy of determining points F and C by decreasing velocity of coagulation

From Fig 27 the following points may be noted: (1) The duration of the period of fibrin formation is relative, varying with the concentration of the recalcifying solution as well as with the concentration of the anticoagulant solution. (2) The minimal duration of fibrin formation coincides with the maximal coagulant effect of calcium for the specimen of that particular anticoagulant solution. (3) The duration of the period of the formation of fibrin is increasing with increasing concentration of anticoagulant solution. (4) In a specimen of a given concentration of anticoagulant solution, the duration of the period of fibrin formation is increasing with concentrations of the recalcifying solution falling below and above the maximal coagulant effect of calcium for that particular specimen

the anticoagulant and the coagulant solutions. (2) In the same sample of citrated plasma the relatively maximal quantity of fibrin is produced by recalcification with quantities of calcium to a certain extent exceeding the maximal coagulant effect of calcium for that particular specimen. (3) At quantities of calcium below and above this value there is a reduction in the relative quantity of fibrin for this particular specimen. (4) The relative maximal quantity of fibrin is increased with reducing concentrations of anticoagulant solution. (Fig. 29.)

I have been somewhat hesitant in presenting these latter observations. I considered them a warning of the possible fallacy of my experimental setup. This necessitated recapitulation and repetition. By adding a solution of thrombin to "serum" expressed from the clot after completion of the regular test,

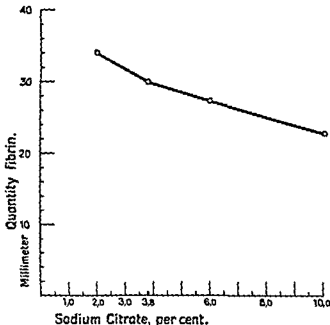


Fig 29.—Variability in the maximum quantity of fibrin formation in relation to variations in the concentration of the anticoagulant solution (9 parts of whole blood to 1 part of solutions of sodium citrate of 2 0, 3 8, 6 0, and 10.0 per cent, respectively). The four specimens were all obtained from the same individual

I could obtain a definite impression of whether I was actually dealing with serum in the physiologic sense of the word or still being confronted with a liquid containing fibrinogen By optimal recalcification of plasma of low anticoagulant concentration (9 volumes of blood to 1 volume of 2 0 per cent sodium citrate), no additional fibrin formation could be found by the addition of thrombin to the liquid expressed from the clot. This was occasionally the case when a somewhat higher concentration of anticoagulant solution was used (3.8 per cent) and invariably was the case when concentrations were 6 0 or higher.

Comment.—In certain respects the type of investigative approach employed here is admittedly defective Certain of the results are fully interpreted and

From these observations one may be justified in concluding *that the relative duration of the period of fibrin formation stands in a certain relation to the velocity of the entire process of blood coagulation.*

Variability in the Quantity of Fibrin Formed.—During the investigations of the problems mentioned before, the appearance of the coagelgrams presented certain variations which suggested further studies. These concern the variable factor of the coagelgrams expressing the relative reduction of transmitted light during the period of fibrin formation. It has been shown in a previous chapter how this variation in transmitted light under standardized physical conditions is proportionate to the quantity of fibrin formed during the coagulation of citrated plasma, and how this finding has been utilized in working out a photo-electric method for the quantitative determination of fibrin.

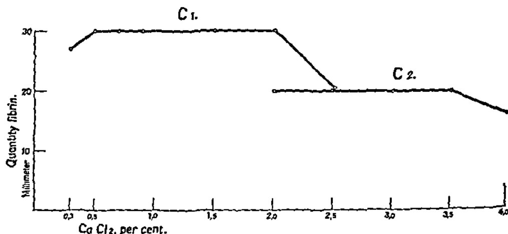


Fig. 28.—Variability of the quantity of fibrin in relation to variations in the concentration of solutions of $CaCl_2$ in identical specimens with varying concentrations of anticoagulant solution (9 parts of whole blood to 1 part of sodium citrate, of 3.8 and 10.0 per cent in specimens C_1 and C_2, respectively)

The coagelgrams of the previously mentioned investigations exhibited, under various conditions, irregularities obviously greater than those to be expected on the grounds of experimental errors alone. Closer investigation revealed a variability in the quantity of fibrin formed by the recalcification of citrated plasma under various experimental conditions.

The technique employed has been identical to the one described The vertical lineary distance in millimeters shown by the coagelgram during the period of fibrin formation has been recorded as the quantitative equivalent of milligrams of fibrin.

In Fig. 28 is illustrated the variability of the quantitative equivalent of fibrin by recalcification with increasing concentrations of calcium, the specimens being from the same patient and simultaneously obtained but with different concentrations of a solution of sodium citrate. Similar observations are made in a good many serial investigations of the same type, all indicating the same trend; namely. (1) The quantity of fibrin formed by recalcification of citrated plasma appears to be relative, varying with the concentration of

10.2 and 5.7 minutes respectively. *It is interesting to note also as regards calcium that we are operating here with a factor not of absolute quantitative meaning.* Ransmeier and McLean, in their attempt to formulate an empirical equation for the determination of coagulation time on the basis of ion activity, have failed to recognize this point to its fullest extent For this reason their equation has only limited applicability so long as the coagulability factor cannot be given an adequate quantitative expression.

This line of reasoning has a reverse side of practical consequence. *Granted that the coagulation time of a specimen under these conditions is varying with the calcium ions of the specimen, it follows that by maintaining a constant ion activity in a series of investigations the resulting variation in the coagulation time can be considered a relative expression of the coagulability of the specimen* This implies among other factors that the investigations are performed at standardized physical conditions

The necessity of standardizing the procedure also as regards the anticoagulant solution follows clearly from a study of Figs. 25 and 26 The results recorded by these illustrations are readily understood on the basis of the conclusions drawn by Nordbö.

Variations due to changes in the pH of the specimens have been considered in view of the investigations of Peretti Changes in the des-ionizing influence of calcium by citrate ions in the light of changes in the pH can hardly be considered an explanation of these results The changes in the pH following the use of anticoagulant solutions of from 3.8 to 10.0 per cent sodium citrate produce variations of the pH of small degrees as compared to those described by Peretti as being of importance for a variation in the des-ionizing influence of citrates (Fig. 26).

In this chapter the velocity of the reaction has been expressed by the coagulation time. As illustrated by Fig. 27 identical results might have been obtained by employing the duration of the period of fibrin formation as a measure of velocity. As previously mentioned, there exists a definite relationship between the duration of the period of dissociation and that of fibrin formation It may be expressed that *the period of fibrin formation is governed by the first stage of the coagulation process* I consider this relationship of essential importance An analysis of the mechanism of blood coagulation will have to include a closer consideration of this point At this stage of the development a complete consideration of this finding is not possible without the support of other findings, which are to be presented later For this reason further comments are reserved. This likewise holds true for the meaning of the variations in the quantities of fibrin.

Standardization of the Technique for the Volume of the Anticoagulant Solution

In the preceding part of this chapter it was concluded that in order to obtain comparable results it was further necessary to include a standardization of the quantity of citrate per volume unit of plasma It is desirable now to consider this point more fully

understood only on the basis of the studies of Nordbö, who has demonstrated clearly the paramount importance of the concentration of ionized calcium in this reaction, both absolutely as well as in relation to the concentration of the electrolytes of the plasma. It is evident that the present investigations would have profited greatly by taking Nordbö's approach into practical consideration. For technical reasons, I have been unable to do so.

Even so, the results may be of some consequence. First, however, it seems desirable to obtain an idea of the dependability of these investigations when *dealing with the calcium factor of blood coagulation.* This may be done conveniently by performing a comparison with previous, more elaborate researches on this subject. For comparison may be chosen the results of the minimal *requirement of calcium for the coagulation of citrated or oxalated plasma.* Nordbö calculated his results on the basis of coagulation of the specimen within 24 hours; Ransmeier and McLean calculated their results by including observations of coagulation times within 100 minutes Even if my observations do not include coagulation times longer than 20 to 25 minutes, it is obvious from the graphs of Ransmeier and McLean as well as from mine that, when the velocity of the reaction is rapidly decreasing, with minute reductions in the quantities of calcium, one is rapidly approaching this minimal value. At present this value is found to approach 1.6 mM. (Fig 23 and Table VII). Nordbö determined this value to be 0.15 to 0.18 mM ionized calcium per kilogram of plasma. *Ransmeier and McLean's figures were slightly higher. Without further mathematics, however, our figure cannot be directly compared with the latter figures It is evident that in our figure only a certain percentage is represented by active calcium ions. It may be fair to assume that if this point is considered our figures may not differ essentially from those of the other investigators.* To avoid misunderstanding, let it again be emphasized that my intention has not been to attempt a substantiation of the investigations of the other workers. I have desired only to use their results as a yardstick for the applicability of my technique to the present problem. The comparison clearly indicates the inability of the present investigative technique to obtain an abso-

[illegible]

A priori, one *might have assumed that the quantitative analysis on this subject* would reveal absolute values modified only by the concentration of the electrolytes of the plasma. The observations recorded in Fig. 24 seem to reveal an additional reservation. *Specimens with high clotting ability seem to require a reduced minimal quantity of calcium compared to those with lower clotting ability.* In view of Nordbö's conclusion this may indicate a relatively higher calcium ion activity of the former compared to the latter specimens. It can hardly be a question of electrolytes alone. *Most likely a biologic factor is here* introduced into an otherwise clear-cut chemical problem. A strengthening of this impression is gained by a review of the figures of Ransmeier and McLean, who found the *minimal quantity of calcium* for the plasma of human beings to be 0.35 mM. and for that of dog's plasma to be 0.24 mM. At the same time they recorded the minimal coagulation time of the two kinds of plasma as

specimens possess an identical inherent coagulability. It should not be taken to indicate that all specimens with a lower or higher hematocrit value than normal react in the manner indicated.

At this point reference may again be made to Fig. 24 in connection with which statements of practical significance were made before. As indicated by the legend, Specimens 4 and 5 exhibit a reduced hematocrit value. On the basis of the above computation and the technique employed, these two specimens *contain less anticoagulant solution as compared with the other specimens* of the group which were all of normal hematocrit values. If corrected on the basis of the hematocrit value and the anticoagulant solution, Specimens 4 and 5 throughout the series actually would have produced longer coagulation times than those indicated. I feel justified in having stated in the foregoing that this point does not essentially change the conclusions drawn from these observations.

The following exposition aims at a standardization of the technique also in connection with the quantitative relation of whole blood and the anticoagulant solution.

With regard to Table VI the figures of this table are based on the employment of 9 volumes of whole blood and 1 volume of the anticoagulant solution with 0 6 c.c *of citrated* plasma *used for individual observations.* In view of the finding that the anticoagulant solution is distributed solely in the plasma, the actual quantity of the anticoagulant solution per 0 6 c c. of citrated plasma may be readily computed when the concentration of the anticoagulant solution is known. Example: Hematocrit value 40 per cent. Using 3 8 per cent sodium citrate, 0 6 c c. of the citrated plasma contains 3 572 mg. sodium citrate (38 x 0.094 — 3 572 mg).

For the sake of convenience it may be desirable to standardize our technique in relation to the quantitative figures corresponding to the hematocrit value of 40 per cent. In other words, irrespective of the varying hematocrit values, our correction aims at operating with 0 6 c c. *of citrated* plasma containing 0 506 c c. of genuine plasma, 0 694 c c anticoagulant solution of 3.8 per cent, *totaling 3 572 mg. sodium citrate per 0 6 c c citrated plasma.* With varying hematocrit values this is possible by varying the original volume of

TABLE VIII

ACCOUNT OF VARYING VOLUME UNITS OF SODIUM CITRATE (3 8 PER CENT SOLUTION) TO BE ADDED TO 9 VOLUME UNITS OF WHOLE BLOOD*

HEMATOCRIT PER CENT	VOLUME UNITS OF SODIUM CITRATE (3.8 PER CENT)
10	1 500
20	1.333
30	1.166
40	1.000
50	0 833
60	0.666
70	0.500
80	0.333
90	0 166

*Irrespective of the hematocrit value, 0 6 c.c. of citrated plasma will contain 0 506 c.c. of genuine plasma, 0 094 c.c. of sodium citrate of 3 8 per cent equalling 3 572 mg

In the foregoing, two works have been considered, namely, those of Nordbö and of Ransmeier and McLean, in which due attention was paid to this point by determination or computation of the electrolytes of the blood. To a certain extent it seems that similar works may have been facilitated by first deciding whether citrates, when added to whole blood, are distributed in the plasma *only or are also diffusing into the corpuscular elements.*

In the available literature I have not been able to find any investigations dealing with this question. It appears to have been taken more or less for granted that an equal distribution of citrates takes place between the plasma and the corpuscular elements On this assumption one would feel justified in standardizing one's technique by adding constant volumes of a fixed concentration of an anticoagulant solution to constant volumes of whole blood. The recent rather extensive investigations dealing with determinations of prothrombin cannot be seen to have had reservations on this point.

In cooperation with Gathe[28] a separate study was undertaken concerning the distribution of citrates in the blood. We proceeded as follows: A definite volume of whole blood was added to a solution of sodium citrate of known volume and concentration. The hematocrit value of the blood was separately determined according to the method of van Allen.[29] The quantity of citrate per volume unit of plasma was determined by precipitating the citrates as pentabromacetone according to the method of Richard,[30] the precipitate being dissolved and titrated according to Kometiani's[31] method. The actual quantity of citrate per volume unit of plasma determined by this procedure was compared to the quantity which would be present theoretically if all the citrate added to the blood remained in the plasma. If all the added citrate was recovered from the plasma, the more complicated analysis of the corpuscular elements as to their citrate content was considered unnecessary. In a small series of cases this was actually found to be so. It was concluded that *citrates added to whole blood remain in the plasma without diffusing into the corpuscular elements.*

Consequently, by withdrawing perhaps 9 volumes of whole blood to 1 volume of an anticoagulant solution, each volume unit of citrated plasma will contain varying volumes of genuine plasma with varying hematocrit values. A computed correlation of these factors was presented in the preceding part of this chapter (Table VI). As was evident from this computation, not only was the actual volume of genuine plasma reducing with increasing hematocrit value, but the actual volume of the anticoagulant solution was increasing both absolutely and relatively. The effect on the coagulation time as caused by variable concentrations of the anticoagulant solution is demonstrated by Figs. 25 and 26 Choosing the normal hematocrit value as 40 per cent and using the correlated figures at this value as a basis of comparison, it is evident that under the conditions stated specimens of lower hematocrit value would exhibit a shortened coagulation time, while specimens of a higher hematocrit value would present a longer coagulation time as compared to that of the specimen of 40 per cent hematocrit. This is, of course, under the condition that the

In order to permit interpolation if such a procedure is required, the figures of Table VIII have been placed in a correlation curve (Fig. 30).

The use of the correction presented here necessitates determination of the hematocrit value before withdrawal of the blood. This may be accomplished either by (1) determination of the hematocrit value of capillary blood or (2) indirect estimation of the hematocrit value through its correlate, the hemoglobin value. The basis of the latter is the finding that the hemoglobin saturation per volume unit of erythrocytes is maintained with surprising constancy, permitting a correlation between the hematocrit and the hemoglobin value. In a previously published report[32] a correlation was worked out between the hematocrit value and grams of hemoglobin per 100 c.c. of blood. By determining the grams of hemoglobin per 100 c.c. of blood, an estimate may be obtained of the hematocrit value This correlation was·

$$\text{Hematocrit per cent} = \text{grams hemoglobin} \quad 2.2136 + 8.47.$$

If determination of the regular per cent of hemoglobin is preferred, the equation of Schartum-Hansen[33] may be employed:

$$\text{Hematocrit per cent} = \frac{\text{Hemoglobin per cent}}{2.6}.$$

Following determination of the hematocrit value by the direct or indirect *procedure, the volume of anticoagulant solution to be added to 9 volume units* of whole blood can now be read directly from the table or the correlation chart (Fig. 30)

In Vitro Changes in the Coagulability of Blood Plasma

As previously stated, the coagulation time of recalcified plasma is prolonged after centrifugation of the specimen, for which reason noncentrifuged specimens must be used

In cases in which there is an increased rate of sedimentation of the erythrocytes, sufficient plasma may readily be obtained within the first hour after withdrawal of the blood. In normal cases, however, this may require a longer time The question remains, how long after withdrawal of the specimen may the blood be examined and still yield dependable results?

In Table IX is indicated the coagulation time of a specimen of recalcified oxalated plasma at varying intervals after withdrawal of the blood. It will be noted in this example, which represents a regular finding, that only small

TABLE IX

IN VITRO PROLONGATION OF THE COAGULATION TIME OF RECALCIFIED OXALATED PLASMA WITH SPECIMEN LEFT AT ROOM TEMPERATURE

HOURS AFTER WITHDRAWAL OF BLOOD	COAGULATION TIME (SECONDS)
1	255
2	240
5	250
7	315
12	430

the anticoagulant solution of fixed concentration. The condition is that a fixed volume of whole blood is retained The possibility remains that the volume of the anticoagulant solution may be kept constant while the volume of whole blood is varied. For obvious reasons the former procedure is preferred. Table VIII records the *varying volumes of 3 8 per cent sodium citrate* to be added to 9 volumes of whole blood at varying hematocrit values, in order to obtain the desired standardization.

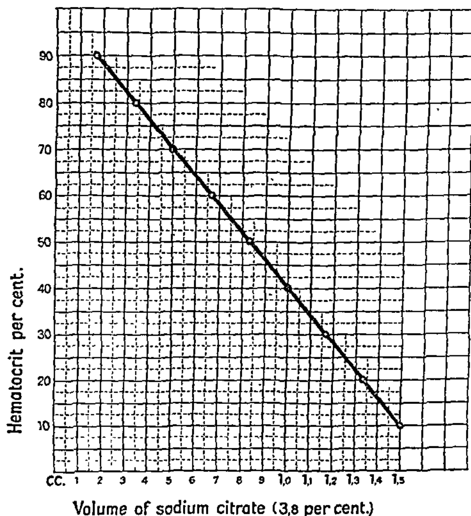


Fig 30—Correlation between hematocrit values, per cent, and the volume of anticoagulant solution of sodium citrate (3 8 per cent) to be added to 9 c.c. of whole blood in order to obtain identical volume and concentration of citrates per volume unit of citrated plasma.

In the procedure indicated here a standardization is obtained on two essential points as is evident *in Table VIII, namely, concerning quantity* of anticoagulant solutions as well as the volumetric interrelation of genuine plasma and anticoagulant solution. *This assures comparable investigative conditions both as regards velocity of the reaction and the quantity of fibrin produced.*

Mechanical Factors Influencing the Coagulation Time of Recalcified Plasma

In discussing the various methods for investigation of the coagulability of whole blood, it was stressed that mechanical influences hastened the velocity of coagulation. I have investigated the effect of similar influences in the coagulation of recalcified citrated plasma.

For this purpose I have chosen, preferably, specimens with marked prolongation of the coagulation time. From such a specimen I would first take a sample and determine the coagulation time of the recalcified plasma according to the regular photo-electric technique; that is, determine the point of maximal velocity of fibrin formation as indicated by the coagelgram. In a second recalcified sample from the same specimen I would read the progress of the coagulation without the aid of the photo-electric technique, in the following manner. introduction of a fine wire into the sample at short intervals in order to determine the point of first formation of fibrin, subsequently tilting the test tube every 15 to 30 seconds to find the point at which a clot formed.

Here is a typical example: The specimen was that from a jaundiced patient with clinical evidence of hemorrhage The coagulation time as read from the regular coagelgram was 880 seconds with point *F* located at about 550 seconds. In the second sample observed visually only, the first thread of fibrin was removed after 245 seconds with the clot formation apparent at 480 seconds The introduction of the wire and the tilting of the tube at intervals had apparently hastened the entire process To verify this suggestion a third sample was recalcified. The wire was introduced into the specimen after 500 seconds, no fibrin was found to have formed After 560 seconds this fibrin formation was well under way. The tube was tilted gently at 645 seconds; the sample was still semiliquid When tilted again at 880 seconds, a solid clot had formed

Repeated similar experiences, particularly clear-cut in specimens with prolonged coagulation time, have revealed the essential advantage of the photo-electric technique for study of the coagulability of blood plasma. *It is, as previously pointed out, a prerequisite for a successful photo-electric recording that the absorption cell with the coagulating specimen remain at complete equilibrium throughout the entire process, thus avoiding hastening the process through mechanical disturbances such as are necessarily associated with any visual observation of the process of coagulation.*

Standard Technique for Determination of the Coagulability of Blood Plasma

To avoid admixture of tissue juices it is a prerequisite that a clean-cut and expeditious venipuncture is performed. A very short venistasis by tourniquet is permissible The blood is drawn into a finely oiled syringe and directly transferred to the graduated test tube containing the measured volume of anticoagulant solution. The volumetric relation between the blood and the anticoagulant solution to be used can be read from Table VIII or Fig 30 (after

variations of the coagulation time occur during the first 3 or 4 hours, with a gradual prolongation during the next 7 or 8 hours. In specimens of citrated plasma the same findings are to be observed, although they are less marked.

As to be shown subsequently, a definite and rather rapid in vitro reduction occurs in the concentration of prothrombin of oxalated or citrated plasma. The fact that this reduction is not registered readily by the coagulation time of the recalcified plasma is easily understood. The content of prothrombin normally represents a large surplus. The present test, like that of Quick for determination of the prothrombin time, answers with a prolongation of the specimen's coagulation time only after a considerable reduction in the quantity of prothrombin.

It is fully possible, also, that other than in vitro changes of the blood may contribute to this prolongation of the coagulation time. In Table X are recorded simultaneous observations of the coagulation time of recalcified oxalated plasma and the in vitro glycolysis.[24] It may be stated that these findings contradict the statements of Stuber and Lang[25] that the anticoagulant effect of oxalate may be explained on the basis of its inhibition of glycolysis

TABLE X

IN VITRO PROLONGATION OF THE COAGULATION TIME OF RECALCIFIED OXALATED PLASMA AND THE DEGREE OF GLYCOLYSIS WITH SPECIMEN LEFT AT ROOM TEMPERATURE

HOURS AFTER WITHDRAWAL OF BLOOD	PLASMA SUGAR (MG. PER CENT)	PLASMA LACTIC ACID (MG PER CENT)	COAGULATION TIME (SECONDS)
½	100	38 8	160
6	79	66 7	180
19	59	127.8	260
48	---	----	420

Without entering into further details as to the nature of the possible in vitro processes of consequence for the coagulability of the specimen, it may suffice to state that it seems permissible, for dependable results, to leave the specimen for three to four hours before performing the recalcification. To counteract the depreciation of prothrombin and the glycolysis that occur, I prefer to leave the specimen in the refrigerator during the period of sedimentation

On the other hand, it is necessary to take into account that shortening of the coagulation time may be brought about through in vitro changes. Occasionally it will be found that a small clot has formed in an otherwise liquid specimen. This is invariably the result of incorrect technique in withdrawal of the blood or the puncturing of the vein The separate clot formation indicates that some conversion of prothrombin has taken place, a fact readily ascertained by an estimation of the concentration of prothrombin, which will be found to be definitely reduced Nevertheless, such a specimen will exhibit marked shortening of the coagulation time of recalcified plasma, a fact which is readily understood when one considers that some thrombin formation has already occurred in the sample. After complete investigation I have made it a rule to check each specimen In the case of small clot formations the readings must be discarded.

TABLE XI

THE COAGULATION TIME OF RECALCIFIED PLASMA IN NORMAL PERSONS

CASE*	COAGULATION TIME (SECONDS)— ANTICOAGULANT: POTASSIUM OXALATE†	CASE*	COAGULATION TIME (SECONDS)— ANTICOAGULANT: SODIUM CITRATE‡
1	170	1	175
2	180	2	170
3	170	3	145
4	120	4	190
5	110	5	155
6	180	6	160
7	170	7	145
8	130	8	160
9	150	9	150
10	140	10	150
11	160	11	180
12	140	12	210
13	150	13	150
14	160	14	170
15	110	15	170
16	200	16	170
17	170	17	155
18	140	18	150
19	150	19	190
20	170	20	130
21	130	21	200
22	200	22	150
23	160	23	170
24	170	24	160
25	150	25	230
26	160	26	230
27	160	27	180
28	190	28	170
29	130	29	180
30	140	30	225
31	140	31	175
32	210	32	215
33	180	33	210
34	170	34	190
35	145	35	170
36	210	36	195
37	180	37	135
38	160	38	180
39	160	39	180
40	190	40	180
41	150	41	180
42	170	42	220
43	160	43	170
44	150	44	155
45	190	45	195
46	170	46	195
47	180	47	230
48	200	48	185
49	120	49	170
50	130		
51	170		
52	160		
53	160		
54	170		
55	160		
56	140		
57	180		

*The results of observations listed in the two columns above were obtained from two different groups of normal persons.

†Average normal: potassium oxalate, 161 ± 21 seconds.

‡Average normal: sodium citrate, 178 ± 28 seconds.

determination of the hematocrit value as previously given). In the earlier investigations I used 1 per cent potassium oxalate as the anticoagulant solution For photo-electric investigations I now prefer the use of a 3 8 per cent solution of sodium citrate ($2C_3H_4OH[COONa]_3 + 11H_2O$).

After gently mixing the blood and the anticoagulant solution the specimen is placed in the refrigerator for sedimentation, following which a part of the supernatant plasma, sufficient for the ensuing investigation, is pipetted off.

Of this plasma 0 6 c.c. is transferred to the absorption cell, which is then placed for 10 to 15 minutes in the water bath, in which is also placed the recalcifying solution ($CaCl_2 + 6H_2O$; 1.0 per cent). When the reactants have attained the desired temperature the test is performed as follows:

The motor of the photelgraph is started, thus assuring an even rotation of the film at the onset of the process. To the plasma in the absorption cell is

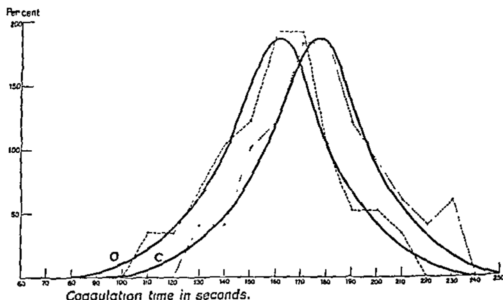


Fig 31 —The frequency distribution curve of the coagulation time of plasma in normals. *O*, Employing oxalated plasma, *C*, employing citrated plasma (see Table VIII).

now added 0.2 c.c. of the recalcifying solution At the moment of addition of calcium chloride, *this moment constituting the zero point of the reaction,* a switch starts the automatic time recorder of the apparatus To obtain rapid mixture of the plasma and the recalcifying solution the absorption cell is shaken gently, after which it is placed in its socket in the water bath. Through the rheostatic arrangement the illumination is now adjusted to the fixed value of 9.5 microamperes of unabsorbed light

These mechanical performances, which usually require about 15 seconds, do not interfere with the accuracy of the reading. The zero point of the reaction is given by the addition of the calcium chloride, from which point the automatic *time recording and process itself is in progress without being in*fluenced by these mechanical manipulations.

trifugation. To obtain comparable results it is necessary to employ noncentrifuged plasma.

Illumination of the Specimen During Photo-Electric Investigations— Within certain limits, *variations in the illumination of the recalcified plasma do not influence the velocity of the process.*

Any variation of the illumination will influence greatly the relative reduction of transmitted light during the period of fibrin formation As indicated, satisfactory reading technique of the coagelgrams depends upon the ready identification of the point of maximal velocity of fibrin formation. For all practical purposes, therefore, it has been found convenient, with the present technical apparatus, to operate with *arbitrarily chosen illumination, fixed at 9 5 microamperes of nonabsorbed light.* The adjustment to this value is accomplished immediately *after* the recalcification of the specimen and the insertion of the absorption cell in its socket in the water bath.

The Recalcifying Solution.—According to the investigations of Nordbö and others, the minimal quantity of ionized calcium necessary for coagulation is dependent upon the concentration of the electrolytes and the species studied. Present investigations indicate a third factor which appears to be of consequence. It is found that *the minimal quantity of calcium required for coagulation decreases with the increasing, inherent coagulability of the specimen.*

For a given specimen of oxalated or citrated plasma the coagulant effect of calcium above this minimal concentration is increasing with increasing concentrations of calcium until it reaches a fairly wide range of concentrations exerting a maximal coagulant effect. *For investigative purposes it is important to choose a concentration of the recalcifying solution within this range of maximal coagulant effect of calcium for that particular concentration of anticoagulant solution.* Above the range of maximal coagulant effect of calcium the velocity of the reaction is decreasing with increasing concentrations of calcium until the recalcifying solution may fail to bring about coagulation; that is, may act as an anticoagulant solution

The Anticoagulant Solution.—Because the precipitation of insoluble calcium oxalate crystals is accompanied by initial changes in the density of the recalcified specimen, it is preferable to work with citrated plasma in investigations employing photo-electric technique

It is found that *a solution of sodium citrate remains in the plasma without diffusing into the corpuscular elements. In order always to be able to operate with specimens containing a constant quantity of anticoagulant per volume unit of genuine plasma, it is necessary to withdraw a constant volume of blood into volumes of an anticoagulant solution varying according to the variation of the hematocrit The volume of the anticoagulant solution is computed on the basis of a hematocrit value of 40 per cent* In the individual case a preliminary determination of the hematocrit value has to be undertaken It is suggested that this determination may be indirectly obtained through an estimation of the hemoglobin value which is an equivalent of the hematocrit After having estimated

The progress of the reaction is followed, as previously described, through the window in the lid of the apparatus. After fibrin formation is completed, the motor is stopped. Duplicate observations should always be made.

After the development and fixation of the film at the end of the day's observations the coagelgrams are read according to the technique previously described.

The Normal Coagulation Time of the Recalcified Plasma

In Table XI are given the results of the coagulation time of recalcified oxalated plasma in fifty-seven normal individuals and of recalcified citrated plasma in another group of forty-nine normal individuals

A statistical computation of these two groups revealed a normal coagulation time of recalcified oxalated plasma of 161 ± 3.1 seconds, and that of citrated plasma of 178 ± 3.6 seconds.

The frequency distribution curves of these two series are given in Fig 31.

In order to obtain an impression of the probable experimental error of the individual observation I have performed a series of identical observations from each specimen in seven individuals The results are given in Table XII. It is to be noted that the standard deviation increases with the average coagulation time of the plasma However, it is found that the relative error for these samples (coefficient variation = $\frac{\text{standard deviation}}{\text{mean}}$ 100) is practically constant (about 10 per cent)

TABLE XII

SERIAL OBSERVATIONS OF THE COAGULATION TIME OF RECALCIFIED PLASMA USING THE SAME SPECIMEN (CITRATED PLASMA)

CASE	COAGULATION TIME (SECONDS) OBSERVATION NUMBER												ARITHMETIC AVERAGE	STANDARD DEVIATION
	1	2	3	4	5	6	7	8	9	10	11	12		
1	80	80	80	75	80	90	92	85	95	90	82		85	6 6
2	118	100	120	85	100	115	110	110	110	95			107	9 0
3	110	125	95	140	135	130	110	125	130	105			121	15.1
4	210	170	190	170	170	150	145	160	170	160			170	17 3
5	200	170	145	170	155	210	190	170	190	180			178	18 9
6	225	180	200	235	200	190	200	200	250	190	200	225	208	20 5
7	*360*	*310*	*330*	*390*	*410*	*325*	*390*	*400*	*350*	*360*	*380*	*345*	*363*	30.4

Summary

With the aid of the photo-electric technique the coagulation time of recalcified plasma under various experimental conditions has been investigated. The following factors have been found to be of consequence:

The Temperature.—*The velocity of the coagulation of recalcified plasma is at its maximum at temperatures of 37° C, the velocity decreasing with lower and with higher temperatures.* It is indicated that this is due, at least in part, to a thrombin activity varying with the temperature

Centrifugation of the Specimen.—*The velocity of the process decreases with the number of platelets as removed from the plasma by fractionate cen-*

velocity of the processes leading to the first formation of thrombin. Further evidence has indicated the constant relation between the duration of the first and the second stages of the process. This is found to be the case irrespective of whether the changes in the velocity have been caused by any of the factors influencing the velocity, as shown above, or have resulted from inherent changes in the coagulability of the plasma itself.

The Relation Between Quantities of Fibrin and the Velocity of the Process.—*The quantity of fibrin as formed by recalcification of the plasma is not an absolute but a relative figure.*

For a given specimen the quantity of fibrin formed through recalcification at the maximal coagulant effect of calcium decreases with increasing concentrations of the anticoagulant solution above a certain minimal level.

For a specimen of a given concentration of anticoagulant solution, a complete and maximal transformation of fibrinogen results through concentrations of the recalcifying solution somewhat exceeding the range of maximal coagulant effect of calcium. Above and below these concentrations there is a decreasing quantity of fibrin formation. These quantitative variations are possibly caused through variations in the production of thrombin. The points mentioned may be of consequence for certain methods dealing with quantitative estimation of fibrin

References

1. Addis, T.: The Coagulation Time of the Blood in Man, Quart. J. Exper. Physiol. 1: 305-334, 1908.
2. Minot, G. R.: The Effect of Temperature Upon the Clotting Time (Prothrombin Time) of Oxalated Plasma With Calcium, J. M Research 33: 503-506, 1916.
3. Gram, H. C.: The Coagulation Time of Citrated Plasma on Recalcination, Bull. Johns Hopkins Hosp. 31: 364 368, 1920.
4. Mosen, R.: Die Herstellung wägbarer Mengen von Blutplattchen, Arch. f. Anat u. Physiol., Physiologische Abtheilung, pp. 352-370, 1893.
5. Lee, R. I., and Vincent, Beth· The Coagulation of Normal Human Blood; an Experimental Study, Arch. Int. Med. 13: 398-425, 1914.
6. Nygaard, K. K.. A Direct Method of Counting Platelets in Oxalated Plasma, Proc. Staff Meet., Mayo Clin. 8. 365 370, 1933.
7. Guthe, Th., and Nygaard, K. K.: The Photoelectric Determination (Photelgraph) of Ascorbic Acid, Chem. & Indust. 57. 1195-1199, 1938.
8. Arthus, Maurice, and Pagès, Calixte. Nouvelle Théorie chimique de la coagulation du sang, Arch. de physiol norm et path. 2: 739 746, 1890
9. Stassano, H., and Daumas, A.· Du rôle double du calcium dans la coagulation du sang et de la lymphe, Compte rend. Acad d. sc. 150: 937 939, 1910.
10. Nordbö, Ragnvald: Über die Aktivitätskoeffizienten der Kalziumionen und der Oxalationen im Plasma und über die Bedeutung der Kalziumionenkonzentration fur die Gerinnung des Blutes, Skandinav Arch. f Physiol. 75 (suppl 11). 1-46, 1936
11. Lebel, H., Schönheyder, F, and Muus, J.: The Relationship Between Coagulation Time of Plasma and the Concentration of Ionized and Un Ionized Calcium, Skandinav. Arch f. Physiol. 78: 179-188, 1938.
12. Ransmeier, J. C., and McLean, F. C.: The Relationship of Calcium Ion Concentration to the Coagulation of Citrated Plasma, Am J Physiol. 121: 488-494, 1938
13. Mellanby, John· The Coagulation of Blood, J. Physiol 38: 28 112, 1908.
14. Heard, W. N.· The Calcium and Phosphorus of the Blood and a Suggestion as to the Nature of the Act of Coagulation, Ibid. 51. 294 317, 1917.

the hematocrit value, the volume of the anticoagulant solution to be mixed with the constant volume of whole blood can be read directly off a correlation chart.

Interrelation of the Anticoagulant and the Recalcifying Solution.—The coagulation time of plasma at a given concentration of the recalcifying solution is dependent upon two factors: (1) the inherent coagulability of the specimen, and (2) the concentration of the anticoagulant per volume unit of plasma. With identical coagulability of the plasma the coagulation time is increasing with increasing concentrations of the anticoagulant solution.

The inherent coagulability of the plasma consequently can be determined by standardizing the technique as to a conveniently chosen, constant concentration of the anticoagulant and the recalcifying solution per volume unit of genuine plasma. As an anticoagulant I prefer to use an isotonic solution of *sodium citrate (3.8 per cent) and in volumes corrected in relation to the varying* volume of the hematocrit, as indicated above The recalcifying solution for the present studies has been a solution of $CaCl_2 + 6H_2O$ (1.0 per cent).

In Vitro Changes.—*An absolutely dependable technique for the with*drawal of the specimen is stressed. The presence of minute formations of fibrin in the specimens must be controlled. If present, the readings are to be discarded, as the coagulation time in these specimens will be definitely shortened as a result of the presence of thrombin.

When the specimens are left at room temperature for any length of time, their coagulability is slowly reduced. Dependable results may be obtained within the first 3 or 4 hours after withdrawal of the blood. The specimens may preferably be left in the refrigerator during the period required for sedimentation of the erythrocytes.

The coagulation time of recalcified plasma can be shortened artificially by tilting the test tube at frequent intervals during the process of coagulation. It is a prerequisite for photo-electric registration and recording of the process *that the test tubes and the specimens remain at complete equilibrium during* the entire reaction. *The photo-electric technique thus makes it possible to avoid the error of mechanical interference with the process that is unavoidably associated with the regular visual observation of the coagulation time of recalcified plasma*

Standard Technique.—An outline is given of a routine procedure for determination of the coagulability of blood plasma, standardizing the technique as to the various factors mentioned above

The Normal Coagulation Time.—With this standardized technique the coagulation time of recalcified oxalated plasma of normal persons was found to be 161 ± 3.1 seconds and that of citrated normal plasma was 178 ± 3 6 seconds.

The probable error of a single observation was found to increase with the increasing coagulation time of the specimen.

The present *investigations further have revealed findings primarily of* physiologic interest:

The Duration of the First and the Second Stages of the Process.—As previously stated, the velocity of the process of coagulation is governed by the

CHAPTER VII

THE INTERACTION OF THROMBIN AND FIBRINOGEN

Introduction

As previously mentioned, the actual process of coagulation is represented by the transformation of the protein fibrinogen, pre-existing in the blood in soluble form, into the protein fibrin. Chemically both proteins are essentially identical. By some investigators (Stuber and Lang[1] and others) this transformation is presumed to be accompanied by the formation of thrombin, the latter being conceived as an accessory product of the process. This conception seems definitely to have been discredited. It is generally considered that the formation of fibrin is the result of the interaction of thrombin and fibrinogen The chemical nature of thrombin is still unknown.

Controversy still exists concerning the nature of this reaction of thrombin with fibrinogen. The present status of the problem may be summarized in the words of Klinke[2]. "*Rein theoretisch sind bei einem solchen Fällungsvorgang eines Kolloides wie es bei der Fibringerinnung vorliegt folgende theoretische Möglichkeiten vorhanden. 1. Isoelektrische Flockung (isoionische Flockung). 2 Entladungsflockung. 3. Dehydrationsflockung. 4. Flockung durch chemische Umwandlung (Analogie: Hitzedenaturierung)—Es ist unmöglich sich nach den bisherigen Befunden uber die Veränderungen die sich am Fibrinogen bei seiner Umwandlung zu Fibrin abspielen sichere Vorstellungen zu machen.*"

The prevalent opinion has been that thrombin acts as a proteolytic enzyme. During later years much work has been done indicating a stoichiometric combination between fibrinogen and thrombin. The question appears to be very complex, particularly because the reaction is greatly influenced by the physical change of the reacting system during its coagulation. It seems definitely proved that thrombin can transform at least 200 times, probably much more, than its own quantity, further that at the beginning of fibrin formation a quantity of thrombin disappears, this quantity exceeding several times the minimal quantity necessary to form the amount of fibrin produced (Eagle).[3] With the present incomplete evidence it seems necessary to consider the action of thrombin being of enzymatic or stoichiometric nature as still unsettled.

In preparing the present chapter I have entertained no hopes that my investigations may be of any direct consequence for the theoretic problem mentioned above For this reason any detailed review of the existing literature on this subject has been omitted.

My intention is twofold. During the course of the subsequent exposition I expect satisfactorily to indicate the applicability of the photo-electric technique to problems of practical and theoretic importance concerning the present reaction The second aim is to form a basis for further investigations concern-

15. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med. 13: 76 95, 1914.
16. Smith, C. H.: Studies in Calcium and Blood Coagulation (With Special Reference to the Use of Salted Plasma as a Method of Estimating Clotting Time), Am. J. M. Sc. 169: 572-583, 1925.
17. Nygaard, K. K.: Coagulability of Blood Plasma; Remarks on the Technic of Its Determination, Proc. Staff Meet., Mayo Clin. 9: 150-156, 1934.
18. Von Zarday, I.: Blutgerinnungsstudien (Kalkproblem und allgemeine Salzwirkung), Folia haemat. 52: 33 39, 1934.
19. Horne, R. M.: The Action of Calcium, Strontium and Barium Salts in Preventing Coagulation of the Blood, J. Physiol. 19: 356-371, 1896.
20. Ferguson, J. H.: The Blood Calcium and the Calcium Factor in Blood Coagulation, Physiol. Rev. 16: 640-670, 1936.
21. Sabbatini, L.: Quoted from Ferguson, J. H.[20]
22. Lehmann, Jörgen: Über die Hemmungswirkung der Barium-, Strontium- und Calciumchloride bei der Blutgerinnung, Skandinav. Arch. f. Physiol. 42: 35-42, 1922
23. Buglia: Quoted from Lehmann, Jorgen.[22]
24. Nordbö, R., and Scherstén, B.: Bestimmung der Zitronensäure im Blut mit der Thunbergmethode und mit der Pentabromazetonmethode, Skandinav. Arch. f Physiol. 63: 124-132, 1932
25. Sjöström, Per: Der Citratgehalt im Blutserum als Diagnosticum bei Krankheiten der Leber und der Gallenwege; eine methodologische, tierexperimentelle und klinische Studie, Acta chir. Scandinav. 79 (suppl. 49). 412 pages, 1937.
26. Hastings, A. B., McLean, F. C., Eichelberger, Lillian, Hall, J. L., and Da Costa, Esther: The Ionization of Calcium, Magnesium, and Strontium Citrates, J. Biol. Chem. 107: 351-370, 1934.
27. Peretti, G.: Influenza del pH sulla reazione fra radicale citrico e Ca-ione, Boll. Soc. ital, biol sper 7: 130-133, 1932, abst. Ber. ges. Physiol. 69: 621, 1933.
28. Gathe, R., and Nygaard, K. K.. The Distribution of Citrates When Added to Whole Blood, Skandinav. Arch. f. Physiol., in press.
29 Van Allen, C. M.: An Hematocrit Method, J Lab. & Clin. Med. 10: 1027-1040, 1925.
30. Reichard, O. Die quantitative Bestimmung der Citronensäure als Pentabromaceton in wässerigen Säurelösungen in Citraten, in Fruchtsäften und in Fruchsaftzubereitungen, Ztschr. f. anal. Chem. 99: 81-96, 1934; Die quantitative Bestimmung der Citronensäure in Milch und Käse nach dem Pentabromacetonverfahren, Ibid. 99: 161-169, 1934.
31. Kometiani, P. A.: New Iodometric Method for Determining Citric Acid, Ibid. 86: 359-366, 1931, abst. Chem. Abst. 26: 669-670, 1932.
32. Nygaard, K. K., and Duxbury, Doris L.. The Percentage of Hemoglobin Compared to the Volume of Erythrocytes, J. Lab. & Clin. Med 20. 767-772, 1935
33. Schartum-Hansen, Hall: Hämoglobinbestimmung durch Hämatokrit (Indirekte Hb.-Bestimmung), Folia Haemat. 54. 22-26, 1935.
34. Evans, C. L.: Acid Production in Shed Blood, J Physiol. 56 146-156, 1922.
35. Stuber, Bernhard, and Lang, Konrad: Die Physiologie und Pathologie der Blutgerinnung, Berlin, 1930, Urban & Schwarzenberg, 91 pages

Upon point F occurs a sudden, marked reduction of transmitted light. The tracing falls off steeply at first, then gradually flattens out exponentially, taking a perfectly horizontal course only a considerable time after onset of the reaction (½ to 1 hour). Occasionally, when using citrated plasma, a relative increase in the transmitted light due to retraction of the clot may be noted after a few minutes (Fig. 33).

As in the other types of reactions considered in the present work, the clot is formed after production of a definite minimal quantity of fibrin, this quantity being identical from sample to sample when constant volumetric relations are maintained. As far as time is concerned, this point of clot formation will depend upon the velocity of fibrin formation. A direct impression of this velocity may be had by studying the tracing after point F, from which point the tracing directly represents the velocity curve of fibrin formation.

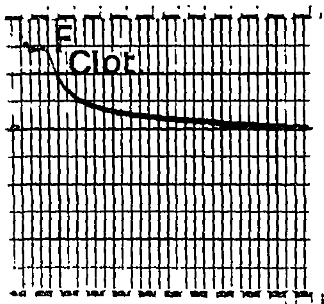


Fig 32.—Photo-electric tracing of the reaction thrombin-fibrinogen. To be read from left to right. First formation of fibrin occurs at point F. The interval from beginning of the reaction to point F is the latent period. Interval between each vertical line on the film is 10 seconds.

A constant finding in thrombin coagulation of fibrinogen is that the relative maximal velocity of fibrin formation is found right after the first formation of fibrin, that is, at the point of relative maximal concentration of the two reactants, thrombin and fibrinogen With reducing concentrations of the reactants the velocity of fibrin formation decreases exponentially as explained above. Under otherwise comparable conditions a further constant finding is that the actual velocity of fibrin formation, measured at its maximal value, is decreasing with decreasing concentrations of thrombin This point is to be considered later in more detail A natural consequence of this fact is that with reducing velocity

ing the coagulation of the blood brought about by activation of prothrombin to thrombin and the practical consequences of this reaction.

Reagents.—The thrombin used in these investigations has been prepared through the courtesy of Tage Astrup, Copenhagen, according to Bleibtreu's[4] modification of the method of Mellanby. The dried thrombin has been conserved in sealed ampules containing measured quantities. A solution of thrombin is obtained by preparing a given amount of dried thrombin with a stated volume of a physiologic saline solution. Commonly the relation has been 0.10 mg. thrombin to 5 c.c. saline solution For the sake of convenience such a thrombin preparation may be termed 100 per cent. It is no solution in the regular sense of the word, as most of the dried material remains undissolved. The solution has been prepared freshly each day to avoid possible reduction of potency through storing.

Two types of fibrinogen solutions have been employed; namely, regular citrated plasma and a prothrombin-free fibrinogen solution. The citrated plasma has been obtained by withdrawing 9 volumes of human blood and adding it to 1 volume of sodium citrate (3.8 per cent).

The fibrinogen solution has been prepared as follows: From a horse, cattle, or sheep 450 c.c. blood is withdrawn into 50 c.c. of sodium oxalate (1.0 per cent). To 240 c.c. of the supernatant plasma obtained after rapid centrifugation is added 80 c c. saturated ammonium sulfate. The rapidly formed precipitate of fibrin is divided from the serum after centrifugation at high speed and dissolved in 240 c.c. of a solution of oxalated saline (0.075 Gm. $Na_2C_2O_4$ in 100 c.c. physiologic saline). The precipitation is repeated twice. The final precipitate is dissolved in 80 c.c. of oxalated saline and dialyzed one hour against oxalated saline solution at low temperature. The stock solution when kept in the refrigerator has been used for 1 or 2 weeks This procedure is the same as that used by Warner and his associates[5] in their work on quantitative prothrombin determinations.

Interpretation and Reading of the Photo-Electric Tracings

The physical basis for the interpretation of the tracings obtained by photo-electric investigation of the present reaction is considered in detail in a previous chapter. The technical procedure of correlating physical changes indicated by the tracings with simultaneously occurring changes in the coagulating system has been identical throughout this work.

In Fig. 32 an illustration is given of a typical tracing of coagulation following the action of a solution of thrombin on fibrinogen

During the first stage of the process no changes of physical consequence occur in the coagulating system. The first formation of fibrin takes place at point *F*. The period of time from the beginning of the reaction to this latter point is termed *the latent period* of thrombin coagulation. This commonly used term is regularly understood to indicate the interval from the zero point to the formation of a clot. No confusion need arise from the application of the term in the sense as used above

tained as an end point. This is demonstrated by Fig. 32. This visual reading technique is less satisfactory with slowly reacting systems in which point F, as demonstrated by the photo-electric tracings, still retains its accuracy.

Quantitative Investigations of the Thrombin-Fibrinogen Reaction

Variation of the Temperature.—The procedure for investigating the effect of the variation in temperature was identical to the one for recalcified citrated plasma. For each observation 2 volumes of the thrombin preparation were added to 6 volumes of the fibrinogen solution. Identical results are obtained whether citrated plasma or the prothrombin-free fibrinogen solution is used The effect of temperature variations on the latent period of thrombin coagulation is given in Fig. 18 *B*. Unequivocal findings indicate that the velocity of the present reaction varies with the changing temperature similar to that noted for recalcified citrated plasma.

In view of the theory that thrombin represents a proteolytic enzyme, particular interest has centered on the determination of the temperature coefficient of thrombin coagulation. Mainly due to the complex nature of the reaction, these investigations have not satisfactorily demonstrated nor definitely refuted the enzymatic nature of thrombin. While a definite optimal temperature is found for diluted solutions, this is increasingly difficult to demonstrate in systems of higher concentrations (Landsberg[8])

The Latent Period in Relation to Varying Concentrations of the Anticoagulant of Citrated Plasma.—Mention has previously been made of the coagulation time of recalcified plasma in relation to varying concentrations of citrated plasma It was found that the coagulation time at maximal coagulant effect of calcium is increasing with increasing concentrations of the anticoagulant solution. These findings were interpreted as resulting from reduced calcium ion activity with increasing concentration of citrate ions in the light of Nordbö's investigations. It was further intimated that a change in pH was possibly of no consequence for the changes observed

Identical specimens of blood employed for the study just mentioned were used in investigations of the present problem The relation of 6 volumes of citrated plasma to 2 volumes of the coagulant solution, in this case a thrombin preparation of 100 per cent solution, was retained The results of this investigation are illustrated by Fig. 34.

A comparison of the results of the investigations in the two different systems (Figs 26 and 34) indicates that increasing concentrations of the anticoagulant solution definitely tend to reduce the velocity of the reaction of both systems In the present reaction the calcium ions do not enter the problem. It is concluded that *the coagulant effect of thrombin is reduced by increasing concentrations of the anticoagulant principle.*

The latter finding does not change the previously presented interpretation of the reduced velocity under identical conditions in recalcified plasma This will be apparent from a study of the actual variations of the time factor. Only a fraction of the variation in the time factor exhibited under these conditions by

of fibrin formation the point of clot formation will lag increasingly behind point *F*, become less and less sharp and therefore less dependable as an end point of the reaction.

A third finding to be further stressed in the subsequent exposition is that, under comparable conditions, a definite quantitative relation exists between the *duration of the latent period* and the relative velocity of fibrin formation. The highest velocity of fibrin appears in reactions with shortest duration of the latent period.

The very slow approach of the tracing to the horizontal level during formation of the fibrin indicates that this process of formation during thrombin coagulation of fibrinogen is very protracted

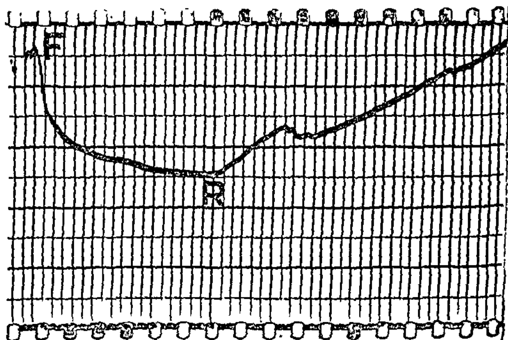


Fig 33 —Tracing of the thrombin coagulation of citrated plasma accompanied by retraction of the clot.

For this reason it is obvious that the present reaction cannot, as was the case with recalcified citrated plasma, conveniently be divided into four well-defined stages. On the basis of the photo-electric tracings of thrombin coagulation of fibrinogen this process may be divided into two main phases: the latent period and the protracted period of fibrin formation. Only in rare cases is a *period of clot retraction observed (citrated or oxalated plasma only).*

Concerning a convenient *reading technique* of these tracings, it naturally follows from the preceding that a convenient end point is represented by point *F*. The practical limits of the accuracy of this end point are to be mentioned in another connection. For reasons just stated it is further clear that in fast reacting systems the point of clot formation may with equal justification be re-

The Latent Period in Relation to Varying Concentrations of Thrombin and Fibrinogen.—Far-reaching deductions and conclusions have been made by workers investigating the quantitative relationship of the thrombin-fibrinogen reaction. Two quantitative determinations have commonly been the subject of study; namely, the duration of the latent period and the quantity of fibrin formed during the reaction. With the visual reading technique as previously commonly employed the end point of clot formation is increasingly difficult to determine with decreasing velocity of the reaction. For the same reason, therefore, a quantitative determination of fibrin formed may not give entirely reliable information on which to base further conclusions. This is particularly the case *when recalling the gradual inactivation of the thrombin with a protracted reaction* On this basis Eagle[3] expressed the opinion that no actual information of consequence for the elucidation of the nature of the thrombin-fibrinogen reaction can be obtained by this investigation. This concept should not be taken as the last word in the discussion. New investigative technique may alter the approach to the problem essentially

Variation in the Concentration of Fibrinogen.—In a series of investigations coagulation has been elicited by adding a 100 per cent solution of a Mellanby-Bleibtreu thrombin preparation to a solution containing decreasing concentrations of fibrinogen. No essential difference is noted whether citrated plasma or a pure fibrinogen solution is employed. The relation between the two reactants was 4 volumes of a fibrinogen solution to 2 volumes of thrombin preparation.

Two kinds of information have been sought by these investigations: (1) the duration of the latent period in reactions with decreasing concentrations of fibrinogen, (2) the relative quantity of fibrin formed under the same conditions during a limited period of observation.

The original tracings from one of these observations are reproduced in Fig. 35 Repeated observations have yielded identical information

Although it may not be too clearly elucidated by the illustration in Fig 35, it is found that there is no essential difference in the latent period from one observation to another in one series This is found to be the case irrespective of the actual concentration of the thrombin in the various serial observations. It is concluded that *the duration of the latent period is not influenced by the concentration of fibrinogen present in the coagulating system.*

In view of Eagle's statement it may be pointed out opportunely that the photo-electric technique as applied to this separate problem avoids any interference from the gelling of the reacting system This physical change occurs *after* the appearance of our end point.

The tracings of Fig 35 clearly demonstrate the second kind of information sought During the period of observation the geometric equivalent of fibrin as measured by the relative reduction of transmitted light during the second stage is decreasing with decreasing concentrations of the fibrinogen. The velocity curve retains its typical form unchanged throughout the series This is what could be expected The relative velocity of fibrin formation remains the same

recalcified plasma may be attributed to a direct inhibition of thrombin activity. It intimates, however, a point of practical consequence. In investigations of the coagulant activity of thrombin the investigative technique must include the maintenance of a constant concentration of the anticoagulant principle if comparable results are to be obtained. As all coagulating systems, no matter of what type, center on the coagulant effect of the thrombin produced, it follows that the *requirement just stated applies directly to all investigative techniques* connected with oxalated or citrated plasma. This also holds true for the estimation of prothrombin, to be considered in a subsequent chapter.

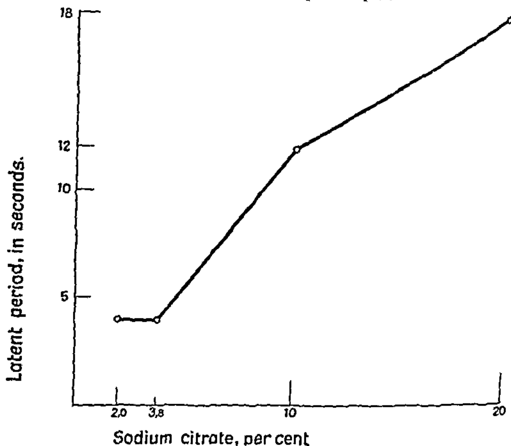


Fig. 34.—Coagulation of citrated plasma by a solution of thrombin of constant concentration (100 per cent). Curve correlates the relation of the latent period to increasing concentration of the anticoagulants of citrated plasma in four specimens from same individual.

In his investigations Ferguson[7] found evidence for the existence of a calcium-containing "intermediary complex" in the formation of thrombin. The quantitative variations noted in the present series of investigations can hardly be explained on an increasing inactivation of this intermediary complex by the anticoagulant solution. Ferguson found this intermediary complex to be influenced by anticoagulants acting directly upon freshly formed thrombin, not upon "ripe" thrombin, however. *Whether this point will influence the velocity of* recalcified citrated plasma requires closer investigations.

when varying concentrations of fibrinogen react with constant concentrations of thrombin. At present I prefer not to present this as a definite conclusion Serial observations with extremely high or low concentrations of thrombin and fibrinogen may indicate important modifications of this apparent rule. A thorough investigation of this point necessarily would have to consider the inactivation of thrombin due to slow reactions or physical inactivation due to rapidly reacting systems. As the nature of the thrombin and fibrinogen reaction is outside the scope of the present work, the consequence of this second kind of information will not be stressed further.

The tracings of Fig. 35 are of interest from another point. It has been indicated previously that the photo-electric technique is applicable to quantitative determinations of fibrin. The tracings demonstrate the basis of this quantitative technique by their decreasing geometric equivalent during the second stage.

With regard to these observations, the constancy of the duration of the latent period with varying concentrations of fibrinogen is of direct practical importance. Innumerable works concerning the coagulability of blood in various clinical conditions have included determinations of the fibrinogen content of the blood. The more dependable investigations have not been able to correlate variations in the quantity of fibrinogen with changes in the coagulability of the blood. In fact, observations have frequently been made, as repeatedly as I have made them, that the fibrinogen may be increased to twice or three times the normal value in spite of severe or fatal hemorrhages in cases of obstructive jaundice In spite of this, textbooks and recent articles still consider the varying amount of fibrinogen produced by the liver a plausible explanation of certain hemorrhagic manifestations In in vitro experiments, at least, the normal content of the fibrinogen of the blood may be diluted fifteen to twenty times and still give a fairly firm clot. *It is concluded that no relation exists between decreased coagulability of the blood and the quantity of fibrinogen except in rare cases of fibrinopenia* In experimental investigation of the function of the liver (chloroform poisoning) a very low production of fibrinogen may be noted in extreme cases Here the picture is confused by the simultaneous impairment of the hepatic function responsible for the production of prothrombin.

Variations in the Concentration of Thrombin—In a series of investigations the concentration of the solution of fibrinogen has been kept constant while the concentration of the thrombin preparation has been reduced by diluting a 100 per cent preparation of the above stated quantities with increasing volumes of physiologic saline solution. The volumetric relation between the two reactants in each test has been the same as described in the foregoing

The actual tracings in a serial observation of this type are reproduced in Fig 36 A composite curve correlating the duration of the latent period with variations in the concentration of thrombin is given by Fig 37

It will be apparent that no direct proportion exists between the concentration of the thrombin preparation and the duration of the latent period.

It is obvious that the appearance of this curve will depend entirely on the potency of the concentration arbitrarily designated as of 100 per cent solution.

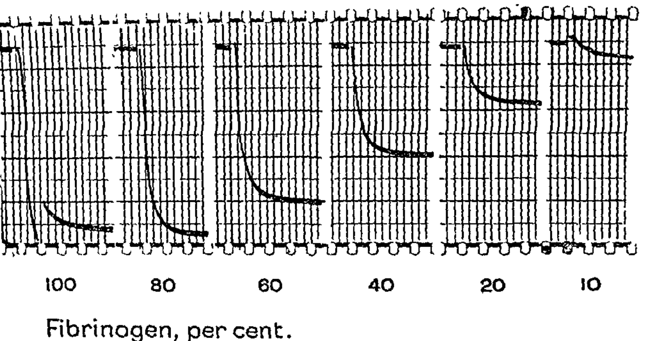


Fig. 35.—A series of photo-electric tracings of the thrombin-fibrinogen reaction indicating the independence of the latent period in relation to the concentration of fibrinogen. The concentration of the thrombin remained constant throughout. A 100 per cent solution of fibrinogen equaled 410 mg. per 100 c.c. solution. The illumination was kept constant throughout the series The beginning of the latent period is to be read from the end of the heavy, upper horizontal line. NOTE: The high concentration of fibrinogen of first sample (100 per cent) necessitated the "breaking" of the tracing in order to get its lower part onto the film.

Whatever potency and concentration of the nondiluted stock solution of thrombin, a series of decreasing concentrations of this preparation will not produce a straight curve between the two correlated values. This is a point of practical consequence as it has been taken more or less for granted by several investigators that such a direct proportion exists. This is a point to be reconsidered in connection with quantitative determinations of prothrombin.

The tracings of Fig. 36 are also of theoretic interest. It will be noted that the tracings produced with a concentration of thrombin of 100, 50, and 25 per cent preparations retain the common pattern with maximal velocity of fibrin formation at the very beginning of the second stage of the reaction The quantity of fibrin formed per unit of time is indicated by the varying velocity with

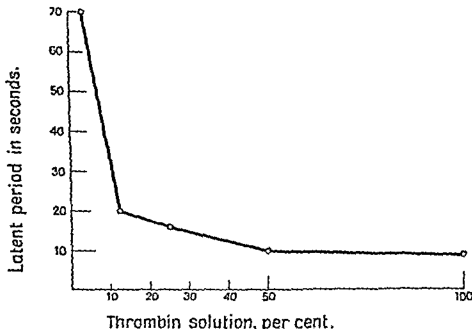


Fig 37.—Curve correlating the latent period with decreasing concentration of thrombin. The quantities are identical to those used in Fig 36 Within 6 hours coagulation did not occur with a concentration below 0 79 per cent thrombin

which the tracing approaches the horizontal base level. The series gives a good illustration of the point frequently stressed also in the section on recalcified plasma, that the velocity of the second stage of the process is governed by the velocity of its first stage

In coagulating systems it appears to be a general rule that the velocity of the entire process is governed by that of its first stage, that is, by the processes leading to the formation of thrombin.

By examining the tracings of Fig 36 produced by the lower concentrations of thrombin, one significant difference is noted between these and those of higher concentrations In specimens exhibiting a somewhat protracted latent

Thrombin
per cent.

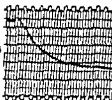
100



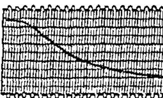
50



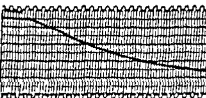
12,5



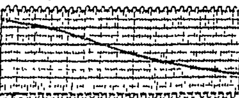
6,25



3,12



1,57



genuine blood exhibits a tracing identical with that of recalcified citrated plasma. In subsequent chapters I hope to stress that the gradual production of thrombin appears to ensure a maximal conversion of fibrinogen into fibrin without any appreciable physical inactivation of the available thrombin The physiologic importance of this mechanism seems obvious

For the reasons here mentioned I must seriously question the following statement by Klinke:[8] *"Thrombingerinnung des Fibrinogens und die Gerinnung rekalzifizierten Zitratplasma können gleichfalls mit demselben Zeitgesetze beschrieben werden, sind also identische Vorgänge."*

Summary

A cursory summary is given of the more prevalent opinions concerning the nature of the thrombin-fibrinogen reaction No direct attempt is made to study the reaction from the enzymatic or stoichiometric point of view.

An interpretation is given of the photo-electric tracings of the thrombin-fibrinogen coagulation, thereby forming a basis for investigations of the quantitative variations of the reaction.

It was found that the first period of the process up to formation of the first fibrin, the so-called latent period, can be employed conveniently as the comparable, quantitative expression for the velocity of the reaction. The valid reason for this procedure is the finding that the velocity of the entire process of coagulation appears to be governed by the velocity of the processes occurring during the first stage of the reaction.

The type of tracing encountered in the thrombin-fibrinogen reaction is characterized by a maximal formation of fibrin at the beginning of the second stage of the process where a relative maximal concentration of the two reactants exists Evidence is presented indicating the autocatalytic nature of the process.

Emphasis has been placed on the difference in this type of tracing as compared to the coagelgrams of recalcified plasma, the appearance of which is explained on the basis of a gradual formation of thrombin. It is pointed out that this difference is of significance for any investigations concerning the experimental study of the process of blood coagulation It is plausible that the existing confusion as to the nature of the process may be due partly to a disregard of these essential differences

The reaction of thrombin and fibrinogen is influenced by the variation in temperature in a way similar to that noted for recalcified plasma

It was observed that *the coagulant activity of thrombin is reduced with increasing concentrations of the anticoagulant solution* No attempt is made to explain this phenomenon With equal justification the expression may therefore be used that in higher concentrations of the anticoagulant solution the fibrinogen complex appears to be more resistant to the coagulant activity of thrombin.

It was found that *the velocity of the reaction is not influenced by the concentration of the fibrinogen in the coagulating system.* The opinion is expressed that, in discussing the pathogenesis of the reduced coagulability of the blood in

period, the first formation of fibrin appears to be slow at first, gathering momentum for a short while and then exhibiting a delayed, relative maximal formation of fibrin. With increasing duration of the latent period this point of maximal velocity of fibrin production increasingly lags behind the onset of the second stage of the process. As may be recalled, this relation was not the exception but the constant finding when dealing with tracings of recalcified plasma.

The full explanation of this phenomenon seems closely linked with the processes occurring during the latent period *At present no explanation can be presented as to how the thrombin changes the fibrinogen into fibrin.* This much appears evident from the present investigation. When relatively maximal concentrations of thrombin and fibrinogen react with each other, the result is the transformation of a certain percentage of the available fibrinogen; further, that this transformation in its turn hastens the subsequent formation of fibrin. By an improved technique of recording details of very rapidly reacting systems, I believe the same findings may be revealed in the higher concentrations of thrombin. *I have interpreted the appearance of the tracings produced by lower concentrations of thrombin as substantiating the suggestion presented by several workers that blood coagulation is an example of an autocatalytic reaction.*

The observations made in the present chapter and the appearance of the tracings presented are of significance for the interpretation of the coagelgrams of recalcified plasma The tracings of the present thrombin-fibrinogen reaction may be taken as a standard type for reactions in which the two reactants, thrombin and fibrinogen, are present in relatively maximal concentration at the beginning of the second stage of the process The tracings of recalcified plasma never exhibit this type of reaction On the contrary, they show a gradually increasing formation of fibrin up to the point of its maximal formation, after which gradual reduction again takes place One can readily assure oneself of the high concentrations of thrombin formed by this reaction. After rolling out of the fibrin already formed the thrombin-containing serum, when added to fibrinogen in identical volumetric relations, as stated in the foregoing, produces a short latent period characteristic of a high concentration of thrombin. The different appearance of the two types of curves consequently must be sought in different methods for the production of thrombin. The coagelgrams of recalcified citrated plasma do not indicate the presence of relatively maximal concentrations of thrombin On the contrary, the coagelgrams appear characteristic of reactions in which a gradual formation of thrombin takes place.

I have wanted to lead up to this point in order to point out that in studies of the kinetics of the process of blood coagulation, the manner in which the thrombin is brought to participate in the reaction is of decisive consequence. *When the preformed thrombin is added to a solution of fibrinogen, the tracings indicate quite a different type of reaction as compared to a system in which the thrombin is formed during the course of the reaction.* A full regard for the essential difference between these two types of reactions reaches beyond a question of great theoretic interest into the understanding of a biologic phenomenon of vital significance It has been intimated previously that the coagulation of

CHAPTER VIII

THE COAGULANT EFFECT OF THROMBOPLASTIN, WITH PARTICULAR REFERENCE TO THE QUANTITATIVE ESTIMATION OF PROTHROMBIN

Introduction

That coagulation of the blood is hastened by addition of tissues or extracts of tissues to the blood appears to have been known a century ago. The subsequent development of this phenomenon has been characteristic of and similar to many of the various problems related to the coagulation of the blood: an accumulation of a large quantity of detailed information, pressing for an adjustment to and incorporation into one or the other of the many prevailing theories of the process. The long and intense study of the mechanism of the coagulant effect of tissue material has of recent years formed a basis of investigations related to a more practical problem as well; namely, that concerning the quantitative determination of prothrombin and its relation to various clinical conditions. A review of a few of the high points of this development will be presented.

According to Pickering,[1] it was observed by de Blainville (1834) that the injection into the blood stream of brain substance, or an emulsion of it, resulted in the intravascular clotting of the blood. At about the same time Buchanan performed a series of investigations (1831-1845) leading to the first clear conception of the clotting as resulting from a transformation of fibrinogen into fibrin. It was a fitting tribute to the importance of these early studies when Gamgee in 1879 took the initiative by reprinting one of Buchanan's articles[2] and called attention to the importance of his work. Buchanan worked with fibrinogen solutions of hydrocele, ascitic, and peritoneal fluids. The addition of blood serum or washed blood clots to these fluids produced coagulation. He likewise noted hastening of the coagulation of diluted salt plasma (sodium sulfate) after adding pieces of fresh lymphatic glands or an aqueous extract of these glands. These observations were shelved for several decades. When Schmidt tackled the same problem, the work of Buchanan appears to have gone unnoticed.

Schmidt (1876)[4] considered that the coagulant effect of tissue material was caused by substances not identical with the fibrin ferment, and he termed these "zymoplastic substances." Schmidt also found these substances to be soluble in alcohol and thermostabile and to contain lecithin. *"Sicher ist es dass unter ihnen das Lecithin in bedeutender Menge vorkommt"* (quoted by Zak[11]). Schmidt announced as his opinion that these substances act by conversion of the precursor of the fibrin ferment into thrombin. Woolridge[6] (1883-1884) noted the coagulant effect of lymph cells or alcoholic ether extracts of lymph cells. This extract was described as consisting chiefly of lecithin and small quantities

various clinical conditions, any consideration of the variation of the concentration of the fibrinogen can be regarded as a nonessential factor. An exception to this constitutes the rare cases of genuine fibrinopenia.

Under otherwise identical conditions, the velocity of the thrombin-fibrinogen reaction is governed by the concentration of the available thrombin. There is no direct linear proportion between the velocity of the reaction and the concentration of thrombin. This relationship has to be expressed by a correlation chart.

References

1. Stuber, Bernhard, and Lang, Konrad. Die Physiologie und Pathologie der Blutgerinnung, Berlin, 1930, Urban & Schwarzenberg, 91 pages.
2. Klinke, Karl: Kolloidchemie der Gerinnung. In Lichtwitz, Leopold, Liesegang, R. E., and Spiro, Karl: Medizinische Kolloidlehre. Physiologie, Pathologie und Therapie in Kolloidchemischer Betrachtung, Dresden, 1934, Theodor Steinkopff, pp. 416-434
3. Eagle, Harry: Studies on Blood Coagulation. II. The Formation of Fibrin From Thrombin and Fibrinogen, J. Gen. Physiol. 18: 547-555, 1935.
4. Bleibtreu, Max: Über Thrombindarstellung, Thrombinnatur, und Thrombinwirkung, Arch. f. d. ges. Physiol. 213: 642-667, 1926.
5. Warner, E. D., Brinkhous, K. M., and Smith, H P.: A Quantitative Study on Blood Clotting; Prothrombin Fluctuations Under Experimental Conditions, Am. J. Physiol 114: 667-675, 1936
6. Landsberg, M.: Studien zur Lehre von der Blutgerinnung Physikalisch-chemische Vorgänge in ihrer Bedeutung für die Thrombinwirkung, Biochem. Ztschr. 50: 245 272, 1913
7. Ferguson, J. F: An Intermediary Complex in Blood Coagulation, Am. J. Physiol. 119. 755-762, 1937.

(60° C.) thus explaining the inactivation of such an extract upon heating, as the coagulating protein removes the phosphatid factor as well. The latter, however, on separate extraction is proved to be thermostabile. A point of great consequence is the finding that, although the cephalin factor alone exerts a coagulant effect, it is in this respect inferior to the aqueous or saline extracts of tissues, in which the cephalin exists in combination with a protein. Most likely it is this latter combination which is the carrier of the coagulant effect of the substances dealt with in the preceding review, a concept previously held by Mellanby[16] (1909). The coagulant effect of this combination deteriorates on standing

Following these reports a great deal of research has been performed, partly dealing with questions related to the technique of extraction and the properties of the thromboplastic material; mostly, however, concerning the importance of the thromboplastic substances in the induction of blood coagulation and their relation to the physiology of the process of coagulation (Howell and co-workers, Bordet and associates, Mills, Pickering, and many others), for which reason they are not further considered here Mention may be made of the tentative conclusion of Ferguson[17] (1936) that the protein compound may in reality be a protein-cephalin-calcium combination in which the protein is a plasma globulin, and the findings of Loeb and co-workers[18, 19] that the coagulant extracts of tissues exhibit a certain degree of specificity for species (1922)

The rather theoretical inclination of the previously reviewed investigations may be said to have branched off into a practical detour by Howell's introduction of a standardized method for the determination of the coagulant effect of thromboplastin. At an earlier date observations had been made relative to the effect of variation of the concentration of the coagulant material on the velocity of coagulation (Mellanby,[16] Zak[11, 12]) A gradual crystallization of this mass of information into a prothrombin test of practical and clinical importance followed during the next two decades

As mentioned previously, Howell[20] (1914), upon finding that the coagulation of recalcified oxalated plasma in cases of hemophilia was markedly prolonged, concluded that this was due to a deficiency of prothrombin. In a subsequent work Howell and Cekada,[21] following a different investigative approach, concluded that in hemophilia prothrombin was present in normal amounts and of normal property It was thereby shown indirectly that recalcification of oxalated plasma could not be considered a measure of the prothrombin content of the plasma Their investigations consisted in the preparation of a solution of prothrombin from normal persons and from patients with hemophilia. These solutions were activated to thrombin by the addition of calcium. When this activated solution was added to a fibrinogen solution it was found that the coagulation time was the same whether the prothrombin solutions from the normal persons or from the hemophiliacs were employed, thus indicating no appreciable variation in the prothrombin content of the two solutions

of fatty acids. *This extract loses its coagulant effect upon removal of the lecithin.* He prepared his extracts from lymph glands. His remark concerning this extract deserves mention: "It should be an emulsion as opaque as milk if the coagulation is to occur quickly."

Halliburton[6] (1888), who confirmed and amplified some of Buchanan's investigations, concluded that the active substance was a globulin which he called cell globulin. In a subsequent work[7] he found that intravascular clotting was produced by what he called nucleoalbumins and contained phosphorus (1892). Here we encounter one of the *first signs of the rapidly developing confusion in* the terminology of these substances. Morawitz[8] (1903) in aqueous extracts of tissues found coagulant substances which were not thermostabile, a finding which produced *no small confusion* in the problem in view of the previous investigations of Schmidt. Morawitz, who considered these substances to represent a kinase, termed the active principle thrombokinase, while Fuld and Spiro[9] (1904) used the term cytozyme, implying it was of an enzymatic nature. This latter term was retained by Bordet and his school but with a somewhat different meaning.

An impetus to further studies was represented by the works of Freund[10] (1910), who concluded that lipoids must be considered of paramount importance for the coagulation of the blood He demonstrated that lecithin, together with calcium, exerts a coagulant effect similar to that represented by the zymoplastic substances of Schmidt, thereby reviving interest in the latter's work on this point

During the next few years important contributions were presented independently by Zak, Howell, and Bordet and their pupils. Zak[11, 12] (1912) found that a lowering of the lipoids of the plasma or a fermentative cleavage of its lipoids resulted in a marked prolongation or complete incoagulability of the blood. This impairment of coagulability was found to be restored by addition of phosphatids prepared, for instance, from brain. He further noted that petroleum ether extract of brain did not produce coagulation alone without the addition of calcium. He concluded that the coagulant effect of lecithin represents a specific activity which cannot be substituted by other coagulant factors of nonlecithin nature. He noted that the clotting time of the blood is shortened with increasing volumes of the extract. Bordet and Delange[13, 14] (1912) showed conclusively that the previously described coagulant substances of tissues in reality are lipoids which exert their effect through the precursor of the fibrin ferment.

Howell[15] (1912) emphasized the necessity of, and described a dependable method for, investigation of the coagulant effect of tissue substances which he, with a term from Wolf, named thromboplastic material or thromboplastin. For convenience this terminology is preferred in the present work as it has been adopted by most of the American workers to whom references are made frequently in this presentation. In ethereal solutions of dried brain or thymus glands Howell found this extract to contain cephalin, which exerts a highly coagulant effect. In aqueous or saline extracts of tissues this phosphatid was found to exist in combination with a protein of low coagulating temperature

likewise verified the low prothrombin level in sweet clover disease as found by Roderick. The blood of patients with hemophilia, leucemia, and thrombocytopenic purpura was found to have a normal prothrombin content.

Several workers who employed Quick's technique have published reports that substantiated these findings. As will be indicated shortly, doubts exist as to the correctness of Quick's contention that the technique he described may be considered a test for quantitative determination of the prothrombin content of the blood. Even so, it seems well assured that his test is of definite clinical value in detecting an abnormal coagulability of the blood resulting from quantitative insufficiency of the mechanism converting prothrombin into thrombin.

The justification of this reservation is presented by Warner, Brinkhous and Smith.[35] They justly maintain that the clotting time as measured by the technique of Quick is a composite of two processes: (1) the conversion of prothrombin into thrombin and (2) the interaction of thrombin and fibrinogen. As stated in their own words: "*The conversion time alone depends in an obscure way upon the prothrombin concentration and upon other variables of unpredictable importance The thrombin phase overlaps the conversion phase* to a variable degree and is itself a complex function of the amount of thrombin formed The uncontrollable summation of these two reactions gives a clotting time which is very difficult to interpret in terms of prothrombin concentration. We feel it is better to separate the two phases experimentally and to use only the time required for the second phase as a measure of prothrombin. To do this we can transform the prothrombin to thrombin in a preliminary step, then the thrombin formed may be titrated by means of serial dilution technique. This technique, used by older workers and very recently by Eagle, permits one to determine the relative potency of various mixtures."

Their detailed technique is considerably more complicated than that of *Quick and can hardly be applied to clinical problems except by institutions supplied with a well-equipped laboratory According to their technique,* thrombin activity is defined as of unitary concentration. One unit of thrombin coagulates a pure fibrinogen solution in 15 seconds. The equivalent prothrombin unit, when completely converted into thrombin, possesses the same activity in relation to the identical fibrinogen solution.

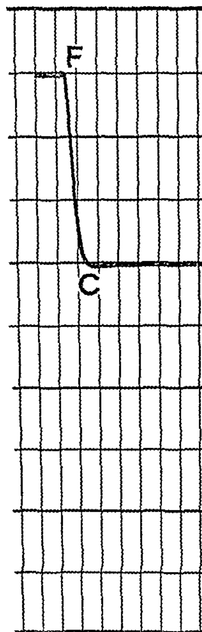
With this technique Warner, Brinkhous, and Smith determined the prothrombin content of the blood of various species. It was found that the blood of mammals contains considerably more prothrombin than that of lower vertebrates *The highest prothrombin level was found in dog plasma; namely,* 350 units of prothrombin, *which level is arbitrarily designated as 100 per cent and in relation to which the prothrombin content of other animals may be expressed* The plasma of normal adult human beings *contains, on this basis,* about 84 per cent with a considerable reduction during early infancy (40 per cent that of adults). The prothrombin level of guinea pigs is about one-half that of dogs

The following investigations have been undertaken to form the basis for a photo-electric study of the coagulant effect of thromboplastic material and its applicability to the quantitative estimation of prothrombin.

In a second series the rate of conversion of prothrombin into thrombin was followed in the two kinds of prothrombin solutions prepared. No appreciable difference was found, thereby indicating that the quality of prothrombin was normal in the blood of hemophiliacs also. An additional observation of essential importance was made; namely, that the coagulation time of a specimen was reduced increasingly with increasing concentrations of prothrombin. A practical basis for a quantitative estimation of prothrombin was formed thereby, although it cannot be seen that this has been followed up by further clinical investigations by Cekada and Howell. This, however, was done in a series of investigations by veterinarians. Roderick[22] (1931) studied the hematologic aspect of cows with symptoms of a hemorrhagic disorder that resulted from feeding them sweet clover hay spoiled in the curing process, the so-called "sweet clover disease." Following the method of Howell and Cekada, Roderick found that prothrombin prepared from cows that had sweet clover disease was ineffective in promoting coagulation, as compared to prothrombin prepared from a normal cow. The addition of small quantities of a cephalin solution to the prothrombin prepared from normal cows resulted in a markedly reduced coagulation time on mixture with the blood, while similar experiments with prothrombin from sick cows revealed a remarkable ineffective coagulation. He further noted a gradual return of the coagulation time toward normal values and a subsequent improvement of the sick animals upon the intravenous administration of a prothrombin solution prepared from normal animals. Roderick concluded that the incoagulability of the blood of animals with sweet clover disease resulted from a reduction of prothrombin.

It is readily understood that the approach presented here, necessitating the preparation of a prothrombin solution of the case to be studied as to its prothrombin level, presents serious difficulties when applied to clinical problems of human beings. This difficulty is overcome by the method of Quick[23] (1935), in which the prothrombin solution and the coagulating system are represented by the patient's plasma. The available prothrombin is converted into thrombin by the addition of thromboplastin and calcium in optimal amounts. The coagulation time, being an expression of the quantity of thrombin present, represents indirectly a measure of the prothrombin converted to thrombin. According to his technique this is obtained by the addition of an optimal amount of calcium and a maximal amount of thromboplastin to oxalated plasma. The thromboplastin is prepared from dried rabbit brain and added in a concentrated solution to the centrifuged plasma before its recalcification. Following recalcification clotting occurs normally between 10 and 20 seconds, varying somewhat with the activity of the various batches of thromboplastic material. The end point of coagulation is determined by inversion of the test tube and visual observation of the sudden gel formation.

With this technique Quick and his associates[24] found a lowered prothrombin content in the blood of patients with obstructive jaundice and degenerative [illegible] to hemorrhage [illegible] rombin. Quick



[illegible] ...ion following re-
[illegible] ...g represents the
[illegible] indicated by the
[illegible] *C* indicates the

Preparation of the Thromboplastic Material

In preparing the thromboplastic material we have followed a technique similar to that employed by Quick.

The brain of a rabbit is carefully freed of the pia and all vessels, following which it is carefully macerated in a mortar. A fresh solution of acetone is poured over the macerated material, which is left in the refrigerator. After 48 hours the acetone solution is changed and left for another 24 hours. If the acetone above the material is not quite clear by this time, it is changed and left for another 24 hours It is then poured off and the material is dried at 37° C.

The fine powder is put up in ampules of colored glass, which are sealed in a vacuum, 0 2 Gm. to each ampule, regularly corresponding to 1.0 or 1.1 Gm. of fresh rabbit's brain. These are kept in the refrigerator and the material retains its potency for many months.

In making up the thromboplastic solution, 0.2 Gm. of the dried material is mixed with 4 c.c. of physiologic saline solution plus 0.2 c.c. of a solution of sodium citrate (3.8 per cent). The mixture is vigorously shaken and placed in a water bath at 45° C. for 10 minutes. After slow centrifugation (500 revolutions per minute for 3 minutes) the opaque liquid is pipetted off. A prolonged centrifugation results in an almost clear liquid which retains only a low coagulant effect In our preparation, at any rate, a good deal of the dried material is not dissolved. It seems, therefore, irrelevant to speak of a 10 per cent solution in this connection. We are no doubt confronted with an emulsion. The strength of this emulsion may be arbitrarily set at 100 per cent, with the quantitative relationship as stated above

The coagulant effect of this emulsion is retained for a considerable time when kept in the refrigerator, but it deteriorates perceptibly within 6 to 8 hours when left at room temperature. For this reason I prefer to make up a fresh emulsion for the investigations to be undertaken in the forenoon and likewise one for the afternoon's determinations The potency of this material has been found to be very high, as will be obvious from subsequent investigations.

Interpretation of the Photo-Electric Recording

Fig. 38 represents a reproduction of an automatically recorded photo-electric tracing of the coagulation that followed recalcification of normal citrated plasma to which had been added an excess of thromboplastin. The tracing is to be read from left to right. Time is indicated by the vertical lines signifying intervals of 10 seconds The zero point of the reaction coincides with the first vertical line

The physical basis for the understanding of this tracing is identical to the one previously described (page 68).

The relative value of transmitted light remains constant up to point *F*, where it is suddenly reduced, giving the tracing an almost vertical direction to point *C* where it again levels off into the horizontal part indicating a lower, fixed value of transmitted light

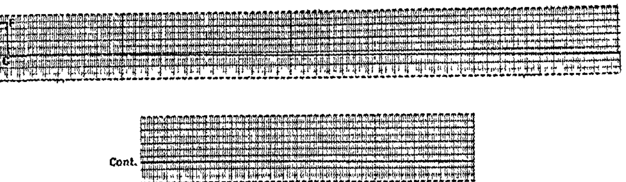


Fig 39 —Photo-electric tracing of the process in a specimen with normal prothrombin time The recording continued for more than an hour Note the very gradual reduction of transmitted light following point G

The investigations of the biologic changes of the plasma leading to these physical changes have been of the same type as previously employed in the interpretation of the coagelgram of recalcified plasma. It has been found that *the point of the tracing indicated by F signifies the point of first formation of the fibrin.*

In order to determine the point of transition of the specimen from a sol to a gel in this tracing, an examination identical to the one illustrated by Fig. 10 has been undertaken. *In the present type of clotting the transition to a gel parallels that of the clotting of recalcified citrated plasma; that is, it is to be found at a point of the tracing placed at approximately the same vertical distance from F as previously described.* The two types of tracing clearly illustrate one essential difference. In the coagelgram of recalcified citrated plasma *the period of fibrin formation is a time-consuming process,* and consequently the transformation to a gel is more gradual. *In the present type of coagulation the second stage of the process is completed normally within a few seconds, of which period about one-third is required for the transformation to a clot.* A phenomenon impressing anyone who has worked with prothrombin determinations is the precipitous change of the specimen from the liquid to the clotted state. A graphic explanation of this phenomenon is clearly illustrated by Fig. 38.

Point *C* of the present type of tracing is not identical to that of plain recalcification of the plasma. In the latter, as will be recalled, *C* indicates completion of fibrin formation In the following, findings will be presented which indicate that point *C* is of a more relative character in the present reaction.

This point has been investigated with the following results.

In a specimen of normal coagulability the recording of the present reaction has been continued for more than an hour (Fig. 39). Point *C* is located about 20 seconds after onset of the reaction. The tracing from this point on reveals a small, gradual reduction in transmitted light. By running control observations before and after, it is ascertained that this reduction is due to changes in the specimen and is not caused by changes in the investigative apparatus In view of the previously presented physical basis of interpretation of the coagelgram, it appears that during this period *we are dealing with a protracted formation of fibrin.*

As will be considered later, the very rapid gel formation is associated with a marked physical inactivation of thrombin It is assumed that this protracted fibrin formation, occurring after point *C*, is an expression of the presence of this thrombin, which may not be completely inactivated after all.

One fact seems evident. Point *C* does not indicate the completion of fibrin formation in the present reaction The expression may rather be used that *point C indicates the relative completion of fibrin formation before the physical inactivation of thrombin sets in.*

Only occasionally will it be seen that retraction of the clot with expression of serum takes place a few minutes after clot formation. Also, for this reason

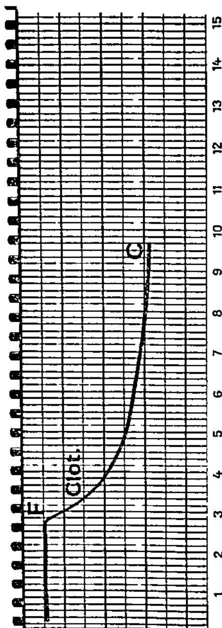


Fig 40.—Photo-electric recording of the process in a specimen with markedly prolonged prothrombin time. The figures below indicate time in minutes. Note the very gradual reduction of transmitted light between points *F* and *C*, indicating the relatively slow formation of fibrin during this stage of the process as contrasted to that of Fig 38.

the previously presented division of the process into four different phases cannot be maintained. We are dealing here with only two stages of the process, (1) from the zero point to the first formation of fibrin, and (2) the period of fibrin formation, which, according to the above observations, is a very protracted process. For the purpose of convenience, however, the second period of the process is to mean in the following exposition the period between points *F* and *C*. Ample reservations will be taken where the biologic changes considered are in conflict with this terminology.

Concerning the fifth variable of this type of tracing, there is no physical reason for a different interpretation of this factor in the present process as compared to that presented before. Ultramicroscopic observations of the entire process are practically impossible because of the rapidity with which coagulation takes place. *In this reaction I have not been able to observe ultramicroscopically the onset of fibrin formation.* This much can be stated. The ultramicroscopic appearance of the fibrin web produced by the present reaction appears to differ in no way from that produced by plain recalcification of the plasma.

Identical tracings are obtained whether oxalated or citrated plasma is used. For the same physical reasons as previously considered (convenience in stabilization of transmitted light), citrated plasma has been employed regularly. The required stabilization to a fixed, arbitrarily chosen value of transmitted light is also performed in these investigations *after* recalcification of the specimen.

The tracing of plain recalcification of plasma differs in this respect from the tracings described in this chapter in that no initial reduction of transmitted light occurs in the latter. This holds true whether the specimens contain a great or a small number of platelets This is taken to indicate that the initial changes of the platelets, as previously described as characteristic of the coagulation of plain recalcification of the plasma, play no essential role when excess thromboplastin is added to the plasma. *This is in accord with the observations* that the number of platelets is of no consequence for the velocity of the present reaction.

Reading Technique

The line of reasoning which forms the basis of the selected reading technique of the coagelgrams of plain recalcification of the plasma is applicable *to the present reaction. As is to* be expected from the previous interpretation, the present possibilities must be somewhat different. This is further obvious by comparing the tracing shown in Fig. 38 with that in Fig. 40. The first tracing represents a reaction of normal velocity; the second, that of a markedly prolonged reaction. *By considering the latter tracing it is evident that it approaches a lower horizontal level only gradually. Point C in this tracing* is geometrically very poorly defined and is excluded as the end point for the reaction.

In all tracings, even those most protracted, the beginning of fibrin formation at point *F* is geometrically well defined and suggests itself as a very

The crucial point in these studies is the potency of the thromboplastic material. How can a constant potency be ensured? Anyone studying the coagulant effect of tissue extracts may have been highly disturbed by the occasionally erratic behavior of some of these extracts. Apparently without any appreciable cause one may obtain an extract of inferior potency. A few times from the same batch of powdered tissue extract I have observed varying degrees of potency of the various ampules. This was found to be only apparent and due to faulty technique in preparing the emulsion. It has been found that, in order to obtain a highly potent emulsion from the powdered material, it must be thoroughly shaken with the required volume of saline solution It was further noted that, with my material at least, the potent emulsion exhibited an opaque appearance of yellowish tinge while a completely milky white emulsion was less potent. In order to learn the potency of this material,

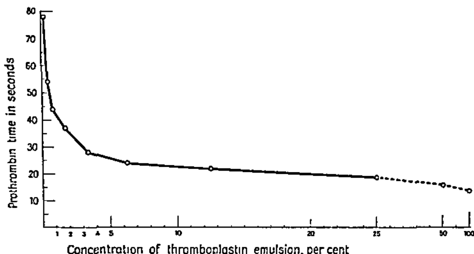


Fig 41 —Prothrombin time of normal persons at varying concentrations of the thromboplastic emulsion.

I have adoped the technique of undertaking, now and then, serial observations of the type shown in Fig 41 A correlation of velocity and dilution of the material are found to present a better check as to the potency of the material than may be obtained by a single observation

By reducing the concentration of the thromboplastin it is mentioned that it may finally be overshadowed by the coagulant effect of the calcium. It is of interest to study the appearance of the coagelgrams in a serial observation of this type The tracings, with decreasing concentration of thromboplastin, approach the type found upon plain recalcification of the plasma (Fig. 42). This suggests a basic similarity or identity between the two types of reactions and will be considered subsequently

The results of the serial observation of Fig. 41 are of particular interest from a theoretic point of view. The problem may be introduced in the form of a question: How can the *reducing* velocity with the *reducing* concentration

convenient end point and has been employed as such in the present work Quick's term, the prothrombin time as expression of the velocity of the reaction, has been retained in the present investigation. *Choosing F as our end point of reaction, the prothrombin time is considered the time interval from the onset of reaction (at addition of calcium chloride) to point F of the photoelectric tracing.*

The two tracings throw additional light on the possibilities of the visual reading technique described by Quick. In normal persons, as mentioned in the preceding chapter, the duration of the present stage of the reaction is very short, explaining the sudden transition of the specimen to a clot. In cases in which the prothrombin time is prolonged, the second stage, as evident from Fig. 40, is a markedly drawn-out process. The point of transition to a clot retains its position in relation to the zero point of the reaction or to point F, although becoming less and less sharp with increasing prothrombin time This does not appreciably detract from the value of the visual reading technique for comparative and practical purposes

Quantitative Investigations of the Reaction

Variation of the Concentration of the Thromboplastic Material.—

Effect on the Velocity in Normal Persons—The thromboplastic material used in these series of investigations is prepared as previously described for a 100 per cent emulsion of thromboplastin In serial observations constant volumes of centrifuged citrated plasma (0 3 c.c), thromboplastin (0 2 c.c.), and the recalcifying solution (0 4 c c. calcium chloride) have been employed, the only varying factor being the concentration of thromboplastin as expressed in percentage of the 100 per cent stock emulsion.

Repeated serial observations of this type have given uniform results (Fig. 41).

No appreciable prolongation of the prothrombin time is noted between 100 and 25 per cent of thromboplastin A most marked prolongation is noticeable below 6 per cent Even with a concentration as low as 0 01 per cent thromboplastin, however, it is noted that thromboplastin exerts a definite coagulant effect. By further decreasing the concentration, one reaches a point where the coagulant effect of the calcium solution equals that of the thromboplastin. It is practically impossible to determine at which concentration this occurs Simple rinsing of the test tube with a weak solution of thromboplastin may still produce a noticeable coagulant effect.

From a practical point of view these observations are of consequence Working with tissue extracts of high potency, a 100 per cent emulsion is far in excess of the critical point of the coagulant effect of the material at around 6 per cent. This guards from erroneous results even with small inaccuracies in the volume of thromboplastin added It is obvious that in order to obtain comparable results a constant relation between the volumes of the interacting factors must be maintained

of thromboplastin be explained? As originally maintained by Schmidt,[4] the mechanism of thromboplastin is explained as a rapid conversion of prothrombin to thrombin. From Fig. 41 two possibilities present themselves:

1. The reducing concentration of thromboplastin may result in increasingly incomplete conversion of prothrombin. This possibility seems ruled out by the presence of calcium, which in itself is able to convert prothrombin to thrombin in the presence of thromboplastin in the plasma (platelets), this ability being far less marked than at the lowest concentration of thromboplastin indicated in Fig 41. A further support of this contention is represented by Fig. 42.

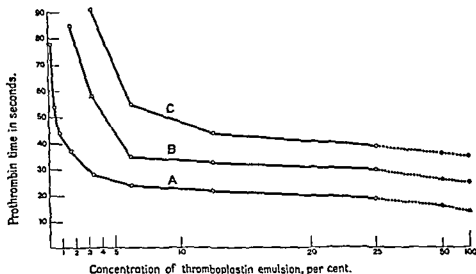


Fig 43 —Prothrombin time at varying concentrations of thromboplastin in specimens of reduced prothrombin content. The lowest curve is that of a normal subject and identical to that shown in Fig 41

2. On the basis that each specimen of the series contains an identical quantity of prothrombin, it must be assumed that we are confronted here with a demonstration of varying velocities of the conversion prothrombin → thrombin. The velocity of the conversion is *reducing* with decreasing concentrations of thromboplastin. This would consequently lead to an increasing period of the actual thrombin formation, with subsequent gradual fibrin formation. That this is actually the case is well illustrated by the appearance of the tracings (Fig. 42). It is concluded that *the concentration of the thromboplastic emulsion governs the velocity of the conversion of prothrombin to thrombin.* It is later to be shown that this is not the only factor governing the rate of conversion

Effect on the Velocity in Specimens With Hypoprothrombinemia—A series of investigations has concerned the effect on the velocity of coagulation by *reducing* the concentration of thromboplastin in specimens with lowered prothrombin content The results of a few of these observations are illustrated in Fig. 43. The more or less parallel tracings support the last conclusion.

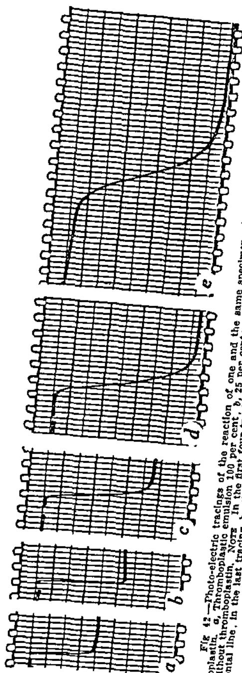


Fig 42 —Photo-electric tracings of the reaction of one and the same specimen with varying concentrations of thromboplastin. *a*, Thromboplastic emulsion 100 per cent, *b*, 25 per cent; *c*, 1 57 per cent; *d*, 0 78 per cent; *e*, recalcification only; without thromboplastin. Note In the first four tracings the *onset* of reaction is indicated by the end of the heavy horizontal line, in the last tracing, by the *beginning* of the film

to any variation in the thrombin of the samples of the series This must be considered constant. The logical explanation seems to be that it is related to the reduced velocity of the reaction, or more specifically to the period of thrombin formation. With increasing duration of this period there is an increasing formation of fibrin up to a point equaling the complete transformation of fibrinogen produced by plain recalcification of the identical specimen (Fig. 42). In other words, the process may be pictured thus: *A very rapid thrombin formation produces a rapid transformation of fibrinogen and subsequent clot production. The still available and free thrombin appears physically inactivated during the rapid clot formation, resulting in an apparent thrombin deficiency, as measured by the fibrin produced. With increasing duration of the period of thrombin formation, a more gradual transformation of fibrinogen will take place, consequently resulting in less and less thrombin being physically inactivated.*

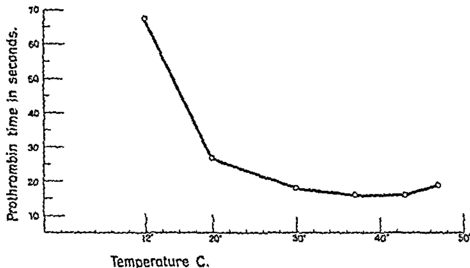


Fig 45.—The prothrombin time in relation to variations in the temperature.

It appears as if the process as characterized by plain recalcification of the plasma implies a mechanism guaranteeing the least chance for physical inactivation of the available thrombin. This is a phenomenon to be considered from various angles during the following résumé It is further of interest in this connection to recall that coagulation of genuine plasma follows the pattern as previously stated to be characteristic of plain recalcification of plasma

Variation of the Temperature.—In investigating the effect of variations in the temperature the same technique has been employed as stated previously. Thromboplastin of 100 per cent emulsion has been used

Effect on the Velocity of the Reaction.—It is found that the velocity of the present reaction is influenced by temperature variations in a way similar to that described for plain recalcification of the plasma (Fig 18). The maximal

The concentration of thromboplastin governs the conversion of prothrombin, whether the prothrombin is present in large or small quantities. The practical consequence is that comparable results of the prothrombin time may be obtained by standardizing the technique at one definite concentration of thromboplastin. Realizing the impossibility of standardizing this emulsion with the accuracy characteristic of chemical solutions it seems only reasonable to maintain a highly concentrated emulsion. In our practical investigations we have, as stated, used a 100 per cent emulsion of thromboplastin

Fig. 43 demonstrates another practical approach for obtaining comparable results in estimating differences in prothrombin quantities. Instead of reading prothrombin times at a fixed concentration of thromboplastin, the reverse procedure may be employed. From Fig. 43 it is apparent that a *fixed* coagulation *time in each of the specimens corresponds to various concentrations of thromboplastin*. Increasing concentrations of the latter are required with decreasing prothrombin values. Considering the standard coagulation time as a summation of the conversion time and the time required for reaction of the thrombin with fibrinogen (the latent period of thrombin), it is obvious that by

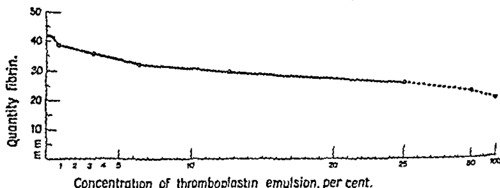


Fig. 44.—The geometric equivalent of fibrin (between *F* and *C*) at varying concentrations of the thromboplastic emulsion. Same specimen as used in serial observations of Fig. 42

reducing the latter to one-half the conversion time must be doubled This is obtained by increasing the concentration of the thromboplastin. This is the basis of the technique as used by Dam and Glavind.[26] It readily explains the increasing quotient *R* found by these authors with decreasing quantities of prothrombin. Although the procedure is sound enough, the necessity of performing a titration with various concentrations of thromboplastin appears to complicate the procedure unduly.

The Fifth Variable in Relation to the Concentration of Thromboplastin.—The geometric equivalent of the relative reduction of transmitted light between points *F* and *C* is determined in the same way as previously described; that is, by measuring the linear vertical distance between the two points.

By performing serial observations with *reducing* concentrations of thromboplastin of the type just described, it is found that the geometric equivalent of fibrin is increasing inversely to the concentration of the thromboplastin (Fig. 44). In view of the concept just presented, this phenomenon is not due

as previously described for plain recalcification of the plasma. After sedimentation of the erythrocytes the supernatant plasma is pipetted off and divided into a number of test tubes which have been centrifuged at varying degrees of speed and duration. Simultaneous determination of the number of platelets has been undertaken. The observations have all given identical results. Centrifugation of the plasma resulting in a reduction of the number of platelets from 400,000 or more to 1,500 platelets per cubic millimeter does not result in any variation of the coagulation time of the specimen. This is to be expected. The actual quantity of thromboplastin, as represented by the platelets, is insignificant in relation to the enormous excess as represented by the addition of a 100 per cent emulsion of thromboplastin. The latter has to be diluted to well below 0 01 per cent emulsion before it is possible to notice any influence of the number of platelets present.

For routine investigations of the prothrombin time we have consequently employed centrifugation of the citrated blood.

The Interrelation of the Anticoagulant and the Recalcifying Solutions

Variation in the Concentration of the Recalcifying Solution.—The blood specimens for these observations have been obtained by taking 9 volumes of blood to 1 volume of trisodium citrate The specimens have all been of normal hematocrit values. The same volumetric relationship between the plasma, the calcium chloride, and the thromboplastin emulsion (100 per cent) as indicated above has been maintained. The only varying factor has been the concentration of the calcium chloride solution. In the serial observations of Figs. 47 and 48 the concentration of the anticoagulant solution was 2.0 per cent sodium citrate

Effect on the Velocity.—The results of this serial investigation are similar to those described for plain recalcification of the plasma (page 107). It is noted that in this reaction the calcium exerts a maximal coagulant effect between 0.3 and 2.0 per cent calcium chloride. Below this range is noted a marked increase in the prothrombin time, with a gradual prolongation above this range. (Fig 47)

With the investigative technique employed, it is evident that the variability of velocity is not due to any variation in the prothrombin content of the plasma For the same reasons as stated in the foregoing it is inferred that we are dealing with a variability in the conversion time of prothrombin following variations in the concentration of the recalcifying solution It is concluded that *the velocity of conversion of the prothrombin is governed by the concentration of calcium ions.*

We thus possess information enabling us to formulate the quantitative relationship involved in the first stage of blood coagulation as expressed by the equation prothrombin + thromboplastin + calcium → thrombin

The two factors, thromboplastin and calcium, determine by their concentration the velocity of conversion of prothrombin. The quantity of prothrombin present determines the quantity of thrombin formed by this conversion.

velocity of the reaction occurs at about 40° C. (Fig. 45). The observation of the rather pronounced reduction of velocity at temperatures below 30° C. indicates that determination of the prothrombin time at room temperatures should be avoided. Prolongation of the prothrombin time of perhaps 10 seconds is of no consequence for the regular methods. For the present method similar prolongation would erroneously indicate definite reduction of prothrombin content if computed on the percentage basis as practiced by Quick. This strongly supports the requirement of maintaining a constant temperature for the observations as set forth by Quick.

In view of previously stated observations it seems clear that the variations of velocity here observed are the result of influences concerning the conversion time of prothrombin as well as of the latent period of thrombin reaction.

By studying the appearance of the tracings obtained during lower temperatures, one observation is of particular interest. The *reducing* velocity of the reaction occurs according to the pattern characteristic of plain recalcification of the plasma; that is, with simultaneous prolongation of the first and the second stages of the process

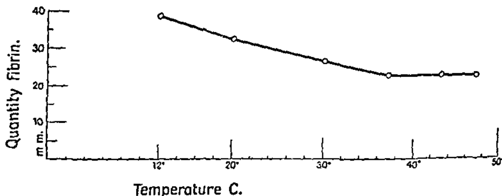


Fig 46.—The geometric equivalent of fibrin (between points *F* and *C*) in relation to variations in the temperature

The Fifth Variable in Relation to Temperature Changes.—In Fig. 46 is illustrated the variation of the geometric equivalent of fibrin as related to variations of the temperature. The observations are taken from the same serial observations as given in the preceding figure

The results are of particular interest as we are dealing here with no qualitative changes of the interacting substances, but are confronted with purely physical variations. One may say that this investigative approach permits a most convenient study of the fibrin production as influenced purely by variations in the velocity of the process. The quantity of fibrin produced between points *F* and *C* increases with increasing duration of the identical period of the process. The tracings are simultaneously approaching the pattern of plain recalcification. This further supports the view presented above.

The Effect of Centrifugation of the Plasma.—In investigating the effect of centrifugation on the velocity of the reaction, we have obtained the plasma

Variation in the Concentration of the Anticoagulant Solution.—In further serial observations recalcification has been undertaken with varying concentrations of calcium chloride of samples of the identical plasma, obtained with varying concentrations of the anticoagulant solution.

An illustration of such a series of observations is given in Fig. 49. As these observations are based on the same type of investigations carried out with plain recalcification of the plasma (page 111), no detailed explanation seems warranted.

The figure illustrates that by recalcification of citrated plasma containing excess of thromboplastin: (1) the minimal prothrombin time increases with

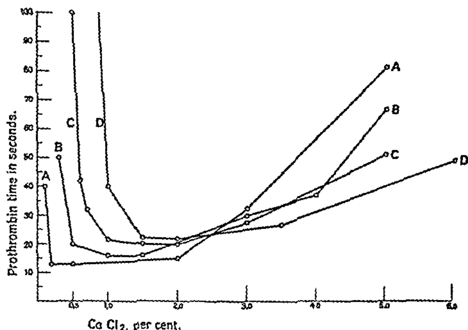


Fig 49.—Variations of the prothrombin time in relation to increasing concentrations of the recalcifying solution in one specimen, to the various samples of which have been added increasing concentrations of the anticoagulant solution To *A*, 2 0 to *B*, 3 8 to *C*, 6 0, and to *D*, 10 0 per cent sodium citrate solution, respectively

increasing concentrations of the anticoagulant solution, (2) the range of maximal coagulant effect of calcium moves toward higher concentrations of calcium chloride with increasing concentrations of the anticoagulant solution; (3) the specimen of the higher quantity of anticoagulant material requires a higher minimal concentration of calcium in order to produce clotting.

On every point this is a repetition of the findings revealed by the same type of investigation carried out with plain recalcification of the plasma. This is a somewhat complicated but nevertheless interesting confirmation of the impression previously gained; namely, that *in recalcification of the citrated or oxalated plasma the physiologic mechanism of the reaction is the same whether*

Effect on the Fifth Variable—An account of the geometric equivalents of fibrin as produced by the addition of varying concentrations of calcium chloride is illustrated by Fig. 48. The explanation of this variability is found in the duration of the first and second stages of the process. We have here another example of the various degrees of physical inactivation of thrombin as resulting from the varying rate of thrombin formation.

Prothrombin time in seconds.

Ca Cl2, per cent.

Fig. 47—Variations of the prothrombin time in relation to increasing concentrations of the recalcifying solution. The anticoagulant solution in this serial observation was sodium citrate (2 0 per cent).

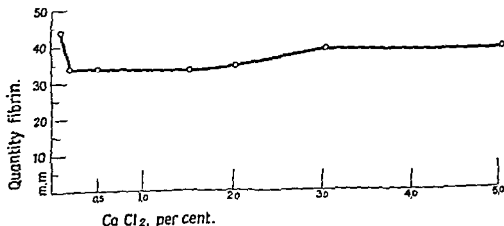


Fig 48—The geometric equivalent of fibrin (between F and C) in relation to increasing concentrations of the recalcifying solution (Same serial observation as Fig. 47).

solutions were coagulated by addition of a pure thrombin solution. It was found that *the activity of identical solutions of thrombin is reduced with increasing concentration of the anticoagulant solution.*

It is concluded that *in citrated plasma containing excess of thromboplastin the recalcification at increasing concentrations of the anticoagulant solutions will produce decreasing quantities of thrombin due to inhibition of the thrombin by the increasing concentration of citrates.*

The above-presented investigations have revealed a point of consequence for the applicability of the present reaction to studies of the prothrombin time and the estimation of quantities of prothrombin of the blood. From the illustrations presented, it is readily understood that the prothrombin time is dependent on the inter-relation of the available calcium and citrate. This necessarily leads to a consideration of the hematocrit value of the individual sample of blood, as considered for plain recalcification of the plasma (page 119). Also in this respect the two types of reactions are identical. It is obvious that the computations, as carried out for the standardization of the technique as to the varying hematocrit values in plain recalcification, are applicable also to this reaction

Granted that such a correction is necessary on theoretic grounds, does this also imply that it is an absolute prerequisite for practical purposes? In order to answer this essential question, reference may again be made to Fig. 50 *A*. The hematocrit value of the specimen examined is 40 per cent. As will be recalled (Table VI), the quantity of anticoagulant solution increases both relatively and absolutely in specimens of increasing hematocrit value. This will naturally in itself lead to a prolongation of the prothrombin time according to the pattern of Fig 50 *A*

The question may be asked In case no correction is undertaken, with which concentration of the anticoagulant solution do we actually operate at varying hematocrit values if standardized on the figures of a normal hematocrit value of 40 per cent?

Table XIII records the result of this computation Finding from the table the actual concentration of the anticoagulant solution with each hematocrit value, an impression may be had of the prothrombin time of a normal specimen at a

TABLE XIII

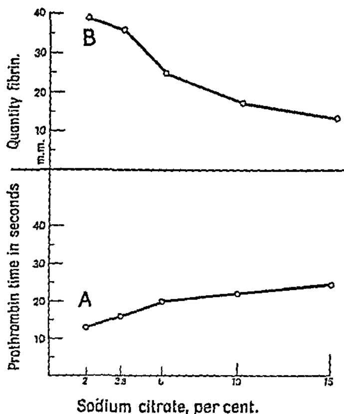
COMPUTATION OF THE ACTUAL CONCENTRATION OF THE ANTICOAGULANT SOLUTION PRESENT AT VARYING HEMATOCRIT VALUES IF NO CORRECTION IS EMPLOYED*

HEMATOCRIT VALUE (%)	ACTUAL CONCENTRATION SODIUM CITRATE (%)
10	2 67
20	2 95
30	3 32
40	3 80
50	4 41
60	5 26
70	6.55
80	8 65
90	12.77

*The basis of computation is the employment of 9 volumes of blood of 40 per cent hematocrit to 1 volume of anticoagulant solution (3 8 per cent sodium citrate).

or not thromboplastin in excess is added to the plasma. In both reactions the concentration of the active calcium ions is of paramount importance for the velocity of the reactions.

Further discussion may be facilitated by considering Fig. 50 *A*. This illustration, simplifying the preceding illustration, records the *minimal* prothrombin time at *maximal* coagulant effect of calcium for each of the varying concentrations of the anticoagulant solutions considered. Simultaneously is recorded the geometric equivalent of fibrin between points *F* and *C* at *maximal* coagulant effect of calcium (*Fig. 50 B*). It is to be emphasized that this value represents the relative minimal value for each of the series



In view of the previously presented findings of the relation between coagulation velocity and formation of fibrin we are here apparently confronted with a paradoxic phenomenon. As is obvious from Fig. 50 the longest coagulation time (at 15 per cent sodium citrate) is associated with the relatively smallest geometric equivalent of fibrin. There is reason to believe that we have encountered here a phenomenon different from the physiologic inactivation of thrombin. In searching for a lead the identical investigative apparatus was employed. Instead of producing coagulation by thromboplastin and calcium, the citrated

solutions were coagulated by addition of a pure thrombin solution. It was found that the activity of identical solutions of thrombin is reduced with increasing concentration of the anticoagulant solution.

It is concluded that *in citrated plasma containing excess of thromboplastin the recalcification at increasing concentrations of the anticoagulant solutions will produce decreasing quantities of thrombin due to inhibition of the thrombin by the increasing concentration of citrates.*

The above-presented investigations have revealed a point of consequence for the applicability of the present reaction to studies of the prothrombin time and the estimation of quantities of prothrombin of the blood. From the illustrations presented, *it is readily understood that the prothrombin time is dependent on the inter-relation of the available calcium and citrate.* This necessarily leads to a consideration of the hematocrit value of the individual sample of blood, as considered for plain recalcification of the plasma (page 119). Also in this respect the two types of reactions are identical It is obvious that the computations, as carried out for the standardization of the technique as to the varying hematocrit values in plain recalcification, are applicable also to this reaction.

Granted that such a correction is necessary on theoretic grounds, does this also imply that it is an absolute prerequisite for practical purposes? In order to answer this essential question, reference may again be made to Fig. 50 *A*. The hematocrit value of the specimen examined is 40 per cent. As will be recalled (Table VI), the quantity of anticoagulant solution increases both relatively and absolutely in specimens of increasing hematocrit value. This will naturally in itself lead to a prolongation of the prothrombin time according to the pattern of Fig. 50 *A*.

The question may be asked: In case no correction is undertaken, with which concentration of the anticoagulant solution do we actually operate at varying hematocrit values if standardized on the figures of a normal hematocrit value of 40 per cent?

Table XIII records the result of this computation Finding from the table the actual concentration of the anticoagulant solution with each hematocrit value, an impression may be had of the prothrombin time of a normal specimen at a

TABLE XIII

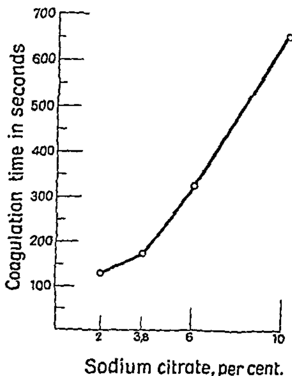
COMPUTATION OF THE ACTUAL CONCENTRATION OF THE ANTICOAGULANT SOLUTION PRESENT AT VARYING HEMATOCRIT VALUES IF NO CORRECTION IS EMPLOYED*

HEMATOCRIT VALUE (%)	ACTUAL CONCENTRATION SODIUM CITRATE (%)
10	2 67
20	2 95
30	3 32
40	3 80
50	4 41
60	5 26
70	6.55
80	8 65
90	12.77

*The basis of computation is the employment of 9 volumes of blood of 40 per cent hematocrit to 1 volume of anticoagulant solution (3 8 per cent sodium citrate).

given hematocrit value by reading the prothrombin time from Fig. 50 *A* at the concentration of the *anticoagulant solution. Considering the prothrombin* time at the normal hematocrit value (40 per cent) as 16 seconds, the same specimen at a hematocrit value of 10 per cent would give a prothrombin time of 14 seconds, at 90 per cent hematocrit value, one of 23 seconds. Applying the percentage computation of prothrombin according to Quick's procedure this would naturally lead to erroneous results

It must be admitted that only rarely are we confronted with specimens in which the hematocrit reaches these extreme values. Most of my observations on the hematocrit value at least fall within the extremes of 20 and 70 per cent.



By referring again to Table XIII and Fig. 50 it is seen that this would indicate a variability of from 15 to 20 seconds prothrombin time, if no correction is made. The variability of the prothrombin time of from 15 to 20 seconds seems of no practical consequence if the percentage computation is not used. For practical purposes *the correction* due to varying hematocrit values may be disregarded when the prothrombin *time* of the blood as such is retained as the relative expression of the prothrombin content of the blood With the present technique and potency of thromboplastin the upper limit of the normal prothrombin time will have to be considered as being about 20 seconds Considering that hemor-

rhages rarely occur in clinical cases before the prothrombin time with the present technique reaches about 50 seconds, the employment of the prothrombin time per se seems indicated. It represents a correlation of relative value. A computation on a percentage basis may too readily erase this important reservation.

In order to take advantage of this line of reasoning, which is identical for both types of reactions, this appendix to the present chapter includes a similar consideration of correction for plain recalcification of the plasma, as presented above for the prothrombin test.

Fig. 51 represents the coagulation time of plain recalcification of the plasma of normal specimens at normal hematocrit values (40 per cent). Otherwise the investigative procedure has been identical to that described for Fig. 50 *A*. A glance at the two illustrations, Fig. 50 *A* and 51, readily brings out one obvious difference: In plain recalcification of the plasma the velocity of the reaction is far more sensitive to variations of the calcium citrate relation than in reactions operating with excess of thromboplastin. This appears to be the essential difference between the two types of reactions. There seems to be no doubt that this quantitative difference is centered on the first stage of the process of coagulation; namely, the conversion of prothrombin to thrombin. The inference is made that *the actual velocity of this conversion is determined by the relation of the quantities of thromboplastin and calcium present at concentrations of maximal coagulant effect of both.*

From a practical point of view these findings are of no small consequence. Assuming both reactions to be of the same clinical consequence, it would follow that the method of practical choice would be the one that necessitates no correction of the anticoagulant solution with the varying hematocrit values. On the basis of the reservation as presented above, it must be concluded that in this respect the prothrombin test is superior to plain recalcification of the plasma.

Variations in the Concentration of Prothrombin.—

Correlation of the Prothrombin Concentration With the So-Called Prothrombin Time.—By addition of fixed volumes of calcium chloride and thromboplastin to samples obtained by increasing dilutions of citrated plasma, information is obtained concerning the prothrombin time in relation to the concentration of prothrombin. The curve of Fig. 52 is the composite of serial observations in twenty-one normal subjects

This correlation curve is found to be practically identical to that of Quick. As he stated, it is evident that normal plasma contains an excess of prothrombin. Dilution of the plasma to about 40 per cent is accompanied by a negligible prolongation in the prothrombin time as compared to the undiluted sample. Under 40 per cent a gradual prolongation to about 10 per cent is noted, below which point a more sudden and marked prolongation occurs Because the fibrinogen is diluted simultaneously, the formation of a regular clot with higher degrees of dilution is increasingly difficult. This is of no basic consequence with the present photo-electric technique whose end point is not formed by the appearance of the clot. In passing it may be mentioned that, if visual readings have to be employed, the difficulty indicated here may be overcome by adding a constant volume of a solution of fibrinogen which together with

a varying amount of saline solution will give the desired dilution. Identical results are thereby obtained as the velocity of coagulation is independent of the concentration of the fibrinogen.

The correlation curve of Fig. 52 is of no small interest. As will be noted, it roughly exhibits a type similar to that correlating the latent period of thrombin coagulation with variations in the concentration of thrombin (Fig. 37). The present curve may be taken as substantiating evidence of the previously considered mechanism of thromboplastin as exerting its activity through a formation of *thrombin from prothrombin It is further evidence of the finding that there is no* straight line relation between the concentration of thrombin and the latent period.

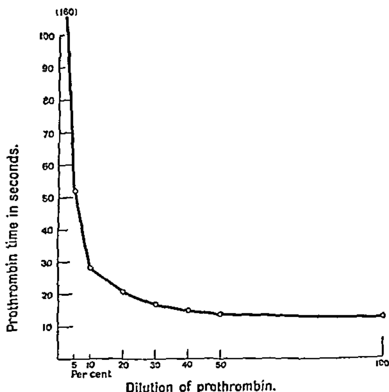


Fig 52.—Curve correlating the prothrombin time at varying concentrations of prothrombin as obtained by increasing dilutions of the plasma. The curve is a composite of observations in 21 normal subjects.

From a practical point of view the present curve attracts particular interest. If it is considered that the data forming the basis of this curve were obtained under favorable experimental conditions, *it is evident that this curve* represents a correlation chart of importance for the quantitative estimation of prothrombin. As apparent from the illustration, the type of the curve makes it less dependable as a correlation chart. As such it can be used with advantage only within a narrow range, perhaps between 5 and 20 per cent. Above this percentage the almost horizontal leg *of the curve does not permit any actual* reading of prothrombin concentrations The best that can be said in this case is that the concentration is not below a stated minimal concentration.

It seems evident that one single determination of the prothrombin time according to Quick's technique is of quantitative significance only in cases of more marked reductions of prothrombin. *Fortunately this reduction is found* in the very group of cases in which prothrombin determinations are of essential clinical importance. As must be clear, the test is not dependable when concerned with smaller variations in prothrombin concentration. One example may clarify

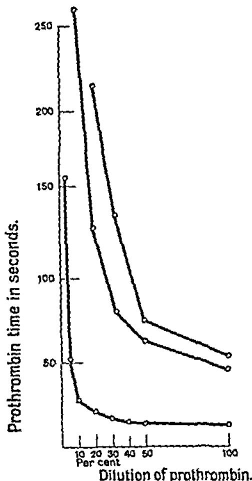


Fig 53 —Curves correlating the prothrombin time at varying concentrations of prothrombin as obtained by increasing dilutions of the plasma. The lowest normal curve is that of Fig 52 The upper curves are from two patients with obstructive jaundice (low prothrombin level).

this point The contention of Smith and his associates that the prothrombin content of the blood in the newborn is about 40 per cent of that of the adult can hardly be verified by the regular technique of Quick solely because of the horizontal part of the correlation chart.

In this connection it seems necessary to touch upon another point of practical interest In clinical reports by Quick and several others employing his

technique, it has been customary to express the results on a percentage basis, considering the adult human plasma as normally containing 100 per cent prothrombin. The percentage is routinely obtained by the following computation:

$$\text{Prothrombin per cent} = \frac{\text{Average normal prothrombin time}}{\textit{Prothrombin time of the patient}}.$$

Example: Average normal prothrombin time is 13 seconds. The prothrombin time of the patient is found to be 26 seconds. According to the above equation, the patient *has a prothrombin concentration of 50 per cent of* the normal adult's.

It is somewhat surprising to see the general acceptance of this method of computation Where quantitative methods are based on a quantitative correlation chart it is the rule that the correlation chart is employed in the generally accepted manner. If this chart is substituted by mathematical computations, it is understood that the quantitative results thereby obtained must conform with those indicated by the chart. It will be seen that such an agreement does not exist in the question considered here. Using the figures as stated in the above example, a prothrombin time of 26 seconds would not indicate a 50 per cent content of prothrombin On the basis of the correlation chart it would correspond to about 12 or 13 per cent.

With specimens of reduced prothrombin level due to pathologic conditions, *serial observations have been performed by dilution of the original plasma* in the manner identical to those illustrated in Fig 52. The results of two such serial observations are given in Fig. 53. It appears that each concentration of prothrombin of the undiluted plasma has its corresponding dilution curve, the latter curve differing increasingly from that of the normal curve, with reducing concentrations of prothrombin, until it approaches the straight line

One-Stage Versus Two-Stage Technique for Quantitative Prothrombin Determination

During the discussion presented above, no reservations were made as to *the validity of the one-stage technique of Quick. As previously touched upon,* this is questioned by Warner and his associates. Investigators contend that quantitative results are obtainable only with a two-stage technique of the nature previously dealt with Because of the rather complicated technique this procedure has not been widely adopted, although it seems generally accepted that it represents at the present the only dependable method for quantitative determination of prothrombin I have wanted to confirm this view by a series of parallel investigations correlating the results of the two-stage technique with those of the one-stage technique performed in identical specimens.

Technique.—Blood specimens were obtained by adding 9 volumes of blood to 1 volume of a 3.8 per cent solution of sodium citrate The specimens were centrifuged and the hematocrit values were determined. The supernatant plasma was divided into two parts Part 1 of the citrated plasma was retained as such. Part 2 was coagulated with 1:10 volume of a freshly prepared

thrombin solution and the fibrin was removed as described in the technique of Warner and his associates. From the remaining serum a series of dilutions was prepared, the saline dilutions of each sample in the series being identical to that of the identically treated citrated plasma. Because of the thrombin coagulation of Part 2, it will be understood that the actual concentration of prothrombin in the series of serum dilutions would be slightly lower as compared to that of the diluted plasma.

For coagulation of the series of diluted plasma a one-stage technique was employed, using the following volumetric relations: 0 3 c c of citrated plasma, 0 2 c.c. of 100 per cent thromboplastin, and 0 2 c c. of a solution of fibrinogen. The onset of the reaction was elicited by the addition of 0.4 c c. of calcium chloride (0.3 per cent).

The samples of the series of serum dilutions were treated according to the two-stage technique. The volumetric relationship and the concentrations of the reactants were otherwise identical to those of the former series. As far as concentrations of prothrombin are concerned, a definite basis for comparison of the one-stage and two-stage techniques may be obtained thereby. Because of the slightly different media of the reaction, it was felt desirable, in addition, to operate with a third series; namely, one of a one-stage technique performed with the serum. This may be obtained readily by addition of the fibrinogen solution to the serum dilutions *before* adding calcium In this third series the relative volumes of the reactants remained unchanged.

Summarizing, we are thus operating with the correlation of varying concentration of prothrombin and the velocity of the reaction, as measured by the following three sets of comparable investigations.

1 The one-stage technique performed with diluted plasma.
2. The two-stage technique performed with diluted serum
3 The one-stage technique performed with diluted serum.

The investigations of the type outlined here have been time consuming As the numbers of one complete observation may have amounted to 30, there is no hope of expressing the results of these studies except by composite curves.

Results—The curves of Fig. 54 are based on the results of serial observations in ten normal adults For convenience normal subjects with practically identical normal hematocrit values have been chosen

One striking fact is the finding that these curves run practically parallel, although exhibiting different levels Of particular interest is the observation that the correlation curve obtained with the two-stage technique is identical in type to that of the one-stage technique, this being the case whether the one-stage technique is performed with the diluted plasma or the serum

This curve (Fig 54 *A*) because of its form is a no more dependable correlation chart than that of the one-stage technique Warner and his associates have not used it as such either Their technique aims at a titration of the specimen with increasing dilutions until a latent period of 15 seconds is reached. The volumetric relations of the interacting factors in the present investigation

have not markedly differed from those of Warner and his associates. It may be justifiable to point out that the selection of 15 seconds as the end point of titration is convenient, as it is located on a sensitive part of the correlation chart.

Following the same line of reasoning for the one-stage technique, it is apparent that equally dependable results may be obtained by performing an identical titration in the citrated plasma or the serum. Due to the different levels of the curves it is evident that the end point of titration for the one-stage technique cannot conveniently be chosen as the time corresponding to the 15 seconds' end point of the two-stage technique. Such a procedure would naturally assure identical quantitative expressions of prothrombin. On the other hand, this would mean an end point falling too high for accuracy on the vertical leg of the correlation chart. With the volumetric relations used here, titration with a one-stage technique as employed in the plasma and aiming at

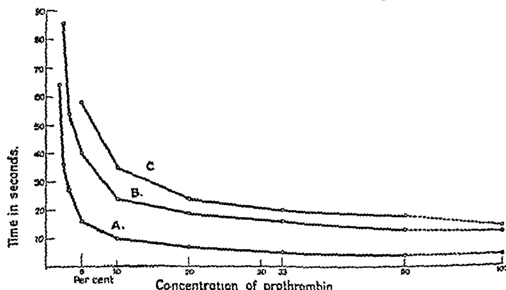


Fig 54.—Curves correlating concentrations of prothrombin with the time required up to first appearance of fibrin in three different coagulating systems A, "Latent period," two-stage *titration technique in serum, B, "serum prothrombin time," one-stage titration technique in serum, C,* "plasma prothrombin time," one-stage titration technique in plasma. Each curve *represents the composite of observations in 10 normal persons*

an end point of titration of 25 seconds for the "serum prothrombin time" and 30 seconds for the "plasma prothrombin time" may be deemed convenient. This is directly apparent from Fig. 54.

It seems clear that the principal objection of Warner and his associates to the one-stage technique can hardly be maintained

Parallel investigations reveal that the one-stage or two-stage technique of prothrombin determination is of equal quantitative consequence, when retaining the principle of titration for both.

This is a somewhat surprising conclusion in view of the theoretic objections to the one-stage technique. In spite of the apparent validity of these arguments, it seems necessary to reconsider them in the light of these experimental findings

The controversy centers on the duration required for conversion of prothrombin to thrombin. According to Warner and his co-workers, this conversion period "depends in an obscure way upon the prothrombin concentration and upon other variables of unpredictable importance"

In the present work evidence has previously been presented concerning the relation of the velocity of prothrombin conversion to the concentration of thromboplastin and calcium. In the present consideration these two factors are kept constant and thus can be eliminated from discussion.

Again referring to Fig. 54, we have three sets of comparable determinations, which together are of consequence in a consideration of the period of prothrombin conversion. Of particular interest are the curves of the one-stage and the two-stage techniques as performed with the same series of serum dilutions It is fairly obvious that the difference in time between identical concentrations of prothrombin in these two composite serial determinations in no way represents an *actual* expression of the duration of the period of prothrombin conversion. However, it may safely be regarded as a *relative* expression of the duration of this period. As such, it is not without meaning. The fact that the two curves run parallel indicates that, while the latent period is changing with varying concentrations of prothrombin, the relative expression of the period of prothrombin conversion remains constant in relation to the first period of the process, as determined by the one-stage technique. On subsequent pages it is to be shown that this holds true also for the actually determined duration of the conversion period in relation to the actually determined duration of the entire first period of the process When this is so, it will be understood that principally no difference of quantitative significance exists between the one-stage and the two-stage technique of prothrombin estimation.

Particulars Concerning the Period of Prothrombin Conversion

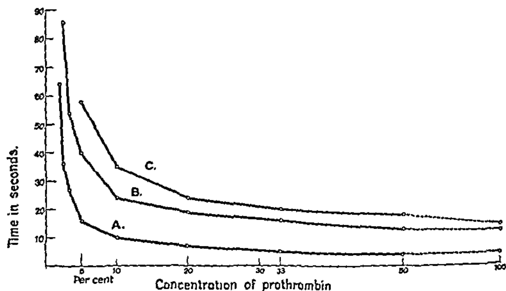
Warner and his associates[27] have indicated that the rate of prothrombin conversion varies in different species. A particularly slow rate of conversion was noted in the guinea pig and in man, a much more rapid one in the dog and the rabbit. These investigators infer that the slow conversion of prothrombin is a contributory factor in the notoriously higher hemorrhagic tendency in man as compared to the dog or the rabbit.

As knowledge concerning the rate of prothrombin conversion is of basic importance in any study of coagulating systems, this problem will be studied separately here This study is limited to the blood of man.

The technique of Warner and co-workers for their study was based on the addition of a solution of oxalate at varying intervals after onset of the prothrombin conversion. Oxalate acts through the inactivation of calcium, thereby arresting at any stage the process of conversion The quantity of thrombin, already formed at the point of addition of oxalate, could be determined in the regular way. Another procedure was to determine the unconverted prothrombin They state that "in plasmas of man and guinea pig 60-75 seconds must

have not markedly differed from those of Warner and his associates. It may be justifiable to point out that the selection of 15 seconds as the end point of titration is convenient, as it is located on a sensitive part of the correlation chart

Following the same line of reasoning for the one-stage technique, it is apparent that equally dependable results may be obtained by performing an identical titration in the citrated plasma or the serum. Due to the different levels of the curves it is evident that the end point of titration for the one-stage technique cannot conveniently be chosen as the time corresponding to the 15 seconds' end point of the two-stage technique. Such a procedure would naturally assure identical quantitative expressions of prothrombin. On the other hand, this would mean an end point falling too high for accuracy on the vertical leg of the correlation chart. With the volumetric relations used here, titration with a one-stage technique as employed in the plasma and aiming at



[illegible] with the time required up to [illegible] *A*, "Latent period," two-stage [illegible] one-stage titration technique in [illegible] technique in plasma. Each curve [illegible]

an end point of titration of 25 seconds for the "serum prothrombin time" and 30 seconds for the "plasma prothrombin time" may be deemed convenient. This is directly apparent from Fig 54.

It seems clear that the principal objection of Warner and his associates to the one-stage technique can hardly be maintained.

Parallel investigations reveal that the one-stage or two-stage technique of prothrombin determination is of equal quantitative consequence, when retaining the principle of titration for both.

This is a somewhat surprising conclusion in view of the theoretic objections to the one-stage technique. In spite of the apparent validity of these arguments, it seems necessary to reconsider them in the light of these experimental findings.

tion. The results may be supplemented by more direct determinations as based on the two-stage technique only, as follows:

The specimens investigated were identical to those referred to before for the two-stage technique After addition of the solution of calcium chloride, the specimens were incubated for varying intervals before addition of the solution of fibrinogen. The latent period was determined. The results of this investigation are illustrated by Fig. 55.

The latent period is dependent upon the duration of incubation. This has been noted by Warner and associates. Their two-stage technique prescribes the determination of the minimal latent period after varying intervals of incubation It appears, however, that they have disregarded the possibility of directly utilizing this finding to study the actual period of conversion.

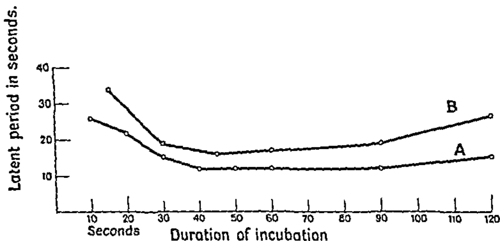


Fig 55 —Curves correlating the duration of the latent period in two samples of different concentrations of prothrombin (A>B) after varying lengths of incubation

From the figure it will be noted that in these particular samples, representing two different concentrations of the same specimen, a short period of incubation is followed by a relatively long latent period. The latter reaches a fixed minimal value after an incubation period definitely longer than that of the first determinations This peculiarity is readily understood. Correct determination of the latent period is not reached before completed conversion of prothrombin If fibrinogen is added to such a system before complete prothrombin conversion, the resultant time factor, appearing as the latent period, will represent a summation of the latent period and the time required for conversion of the still nonconverted prothrombin at the time of addition of fibrinogen. Under these circumstances one would then actually be operating with a combination of a one-stage and a two-stage technique, resulting in erroneous findings.

In Sample A a constant latent period of 12 seconds is reached after an incubation period of 40 seconds, in Sample B the minimal latent period of 15 seconds is reached after 45 seconds. The maximal duration of the period of conversion in these two samples is consequently 40 and 45 seconds, respectively.

elapse before prothrombin conversion is 85-95 per cent complete; in plasmas of dog and rabbit, on the other hand, only 23-30 seconds were required to bring about this degree of conversion." It must be stressed that the time factor determined here, like any time factor concerning velocity of blood coagulation, is only relative and must be seen in connection with the concentrations and volumetric relations of the coagulating systems.

For the present study I have chosen a different approach. This seemed desirable for two reasons. Quantitative interpretation of results is greatly facilitated by the maintenance of a constant volumetric relation between the reactants of the system. The addition of oxalate interferes with this constancy, although it may be corrected by additional computation. In view of the previous findings of the variations in thrombin activity by different concentrations of anticoagulant solutions, I considered it convenient to avoid the addition of oxalate.

In a first investigative approach I proceeded as follows: From a series of normal persons the centrifuged plasma was treated as prescribed for the regular two-stage technique The serum was prepared in a number of saline dilutions ranging from 100 to 10 per cent serum. With each dilution of the series, a test was performed employing the one-stage as well as the two-stage technique. *The concentrations and volumetric relation of the reactants were identical to* those given above

As mentioned before, the difference in time between what may be called the "serum prothrombin time" (one-stage technique in serum) and the latent period (two-stage technique) may be used as the relative expression of the duration of prothrombin conversion. Table XIV presents the average of observations in a series of dilutions in ten normal subjects For each dilution of the series a computation was made of the minimal percentage duration of the period of conversion in relation to the serum prothrombin time That these figures are minimal follows from the fact that the serum prothrombin time is not the direct summation of the period of conversion and the latent period

TABLE XIV

COMPUTATION OF THE APPROXIMATE, MINIMAL PERCENTAGE VALUE OF THE PERIOD OF PROTHROMBIN CONVERSION IN RELATION TO THE ENTIRE FIRST STAGE OF THE PROCESS*

	CONCENTRATION OF SERUM (%)				
	100	50	33	20	10
Latent period (seconds) Two stage technique	4	4	4	7	10
Serum prothrombin time (seconds) One-stage technique	14	14	16	19	24
Percentage minimal value of prothrombin conversion in relation to entire first period of process	71	71	75	63	58

*Computation based on observations on 10 normal human beings.

The objection to this investigative approach is clear, as expressed in the foregoing sentence. However, it retains its relative value as a means of orienta-

factor under investigation, the concentration of prothrombin. I can only agree with the originators of the test that this procedure is sometimes tedious. This does not constitute more than a serious drawback for the practical applicability of the method. The tediousness of its performance might be repaid by accurate results. The fact, however, that the results of the entire two-stage titration technique depend upon the investigator's scrupulous consideration of the varying interval between the addition of the calcium chloride and the addition of fibrinogen constitutes a most serious source of error.

The reliability of the test stands or falls with the dependability and accuracy of the performer of the test. This is my essential objection to the principle of the two-stage technique of quantitative titration of prothrombin. On a very essential point it is practically impossible to standardize the technique because of the personal factor. Admittedly this point is always to be reckoned with in most of our quantitative tests, of whatever nature they may be. When this factor is left to exert its influence on a most crucial point of the reaction, as is the case in the two-stage technique of prothrombin titration, the security of dependable results is definitely shaken. This personal factor may easily be ruled out of its essential place in the technique by prescribing the addition of fibrinogen before the addition of calcium chloride The principle, however, is changed thereby to that of the one-stage technique. The conversion to thrombin and its reaction with the available fibrinogen will then occur during one continuous process, not interfered with by the investigator. No less dependable results are hereby obtained, as are well shown in Fig. 54 and the foregoing discussion. Because the consideration of the duration of incubation can be omitted, the number of tests for one titration is limited to the number of dilutions of the series. In the above example from Warner and associates their twelve tests would be reduced to three In this connection one curious fact may be mentioned During the investigation of the two-stage technique I adopted the procedure of first running a series of tests with the one-stage technique as performed with serum. This has served two purposes, namely, an orientation to the expected duration of incubation and a check on the results of the two-stage technique A well-founded basis for a discussion of the merits of the two principles of quantitative titration of prothrombin is reached thereby. As a consequence of these findings, I am inclined to favor the one-stage titration technique of prothrombin determination.

The Prothrombin Conversion of the Newborn Child

In an investigation of the coagulability of the blood during infancy Owen and associates[23] noted that the two-stage technique only vaguely revealed a transitory reduction of prothrombin as compared to the definite reduction noted by Quick's technique. They concluded that this discrepancy of results was due to the fact that the rate of prothrombin conversion in the newborn is accelerated as compared to that of the adult. I have not been able to ascertain whether this represents an inference or is based on actual investigation of the rate of conversion

The accuracy of this estimation is naturally determined by the number of observations in the series It is further clear that the actual duration of the first period of coagulation (when formation of fibrin first appears) by this procedure can be expressed as a summation of the actually determined duration *of the latent period and the conversion period. In Sample A* it is 52 seconds (40 + 12); in Sample B it is 60 seconds (45 + 15). In the present example it is then found that the percentage duration of the conversion period in relation to the entire first period of the process amounts to 76.9 and 75.0 per cent, respectively, for the Samples A and B. These findings are made repeatedly in identically arranged serial observations. They well substantiate the similar findings, presented above, based on the indirect investigative approach. It is concluded that *in man the period of prothrombin conversion normally covers about three-fourths of the first period of blood coagulation (to formation of the first fibrin).*

The direct approach to the study of the prothrombin conversion as presented above has revealed a point of further interest. By *investigations of* serial dilutions of many sera it has been found that there is a definite quantitative relation between the duration of incubation and the latent period. The incubation for complete conversion of prothrombin is prolonged with increased duration of the latent period. In other words· *The actual duration of the prothrombin conversion is dependent upon the concentration of prothrombin.*

It can now be concluded that *the conversion of prothrombin to thrombin is governed in its velocity by the concentrations of prothrombin, thromboplastin, and calcium.*

The studies related here have uncovered a point significant in the performance of the two-stage method. The accuracy and success of the titration technique of Warner and co-workers will depend upon an accurate consideration of the duration of incubation before addition of the fibrinogen. This point is illustrated by the following example reproduced from the work of these investigators:

TITRATION OF PROTHROMBIN

FINAL PLASMA DILUTION	CLOTTING TIME			
	30 SECONDS' INCUBATION	45 SECONDS' INCUBATION	60 SECONDS' INCUBATION	90 SECONDS' INCUBATION
1·149	14	12	12.5	13
1·223	20	15	15.0	17
1·297	24	21	21.0	23

In this example the arbitrarily chosen standard period of 15 seconds is reached with a dilution of 1.223 after an incubation period of 45 and 60 seconds. The quantity of prothrombin is 223 units per cubic centimeter of plasma. To obtain this result, twelve tests were performed with dilutions at and in the proximity of the correct one, not considering dilutions of higher concentrations Working with a specimen of unknown concentration of prothrombin it is therefore necessary to operate with a great number of tests. No fixed duration of incubation can be decided upon beforehand, as this is governed by the unknown

I considered this relative prolongation of the latent period as an expression of decreasing quantities, or better, increasing inactivation, of thrombin.

The practical consequence of this finding for the performance of the two-stage technique is clear.

What is the physiologic mechanism of this phenomenon? It is a well-known fact that the abundant quantities of thrombin formed during the process of coagulation are completely inactivated by treatment with alkali and subsequent neutralization with an acid. It is not clear why this process of reactivation following inactivation can be successfully performed only a few times. Reactivation may further be obtained by shaking of the serum with chloroform, kaolin, talcum, and other substances, indicating that the reactivation may be brought about by adsorption Furthermore, Landsberg[30] produced inactivation by adsorption of thrombin to casein and serum globulin. From a colloidal point of view, evidence points to the inactivation of thrombin in serum through its adsorption to certain globulins (Klinke[29]). It will be apparent that this is a concept differing from that which considers the inactivated thrombin, the so-called metathrombin, as resulting from a combination of thrombin and antithrombin. Further investigations may decide whether or not there is an essential difference between these two concepts.

Whatever the correct explanation, it seems that this phenomenon of inactivation of thrombin, and the rate at which it takes place, may be studied conveniently by this procedure

Various Methods Possible for the Quantitative Estimation of Prothrombin

The previous exposition has related the influence of various factors on the velocity of coagulation of recalcified plasma containing excess of thromboplastin. It has been stressed that comparable dependable observations are obtained only with an accurately standardized technique

During the presentation of the findings no particular emphasis was placed on the various methods uncovered for a quantitative estimation of prothrombin. A presentation of these methods under a separate subheading may serve a definite purpose Some of them are purely theoretic; others are of practical consequence These are as follows:

1 Quantitative determination of prothrombin based on titration with various concentrations of the thromboplastic emulsion. As mentioned, this is the approach employed by Dam and Glavind (Fig. 43). It is obvious that their arbitrarily chosen, fixed coagulation time of 3 minutes is usable only on the condition that heparin is employed as the anticoagulant Citrated or oxalated plasma cannot be used. From findings given previously it is obvious that in the latter case a coagulation time of 3 minutes would regularly be obtained with only very dilute emulsions of thromboplastin The accuracy of the quantitative results would depend essentially upon the stability of the 100 per cent thromboplastic emulsion. On the other hand, the cost and the variability of the potency of heparin make this procedure less applicable for routine investigations. I

For an understanding of the physiology of prothrombin such a point, when raised, seems significant enough to warrant particular attention. It is taken up in connection with the preceding, as it is of physiologic consequence and not related to the clinical investigations to be considered in a different part of this work.

For this particular study a series of newborn children was selected A computation of the percentage duration of the period of conversion was undertaken for each dilution of the series in each child, the computation being based, as above, on the parallel titrations with the two-stage and the one-stage techniques. The results are given in Table XV. The necessity of performing the computation separately for each dilution of the series is obvious when considering the fact, to be stressed later, that the quantity of prothrombin varies greatly during this stage of life. As noted from the table, the approximate average duration of prothrombin conversion in relation to the entire first period of coagulation does not in this series differ essentially from that previously found for adults.

TABLE XV

COMPUTATION OF THE APPROXIMATE MINIMAL PERCENTAGE VALUE OF THE PERIOD OF PROTHROMBIN CONVERSION IN RELATION TO THE ENTIRE FIRST STAGE OF THE PROCESS*

	THE PERCENTAGE MINIMAL VALUE OF PROTHROMBIN CONVERSION IN RELATION TO THE ENTIRE FIRST PERIOD OF THE PROCESS			
	CONCENTRATION OF SERUM (%)			
CASE	50	33	20	10
1	62	56	50	48
2	82	71	—	60
3	73	63	67	53
4	85	81	73	—
5	77	75	67	61
6	82	70	52	—
7	70	60	60	—
8	73	74	67	—
9	77	75	67	63
10	58	57	50	53
Average percentage.	71	68	61	56

*Computation based on observations on 10 newborn children

These determinations have been supplemented by direct determinations of the minimal latent period after varying periods of incubation and using the one-stage technique, as explained above Identical results were obtained

It is concluded that *the rate of prothrombin conversion of the newborn does not differ from that of the adult*

Inactivation of Thrombin by Prolonged Periods of Incubation

In direct connection with the preceding, a point of some practical and theoretic interest is to be touched upon. In referring again to Fig. 55 it is to be noted that, using the two-stage technique, the latent period is increasing with increasing duration of incubation after it is beyond the range of the minimal level.

withdrawal of the specimen. The result of such an investigation is listed in Table XVI. The particular specimen used in this series was that from a normal person.

TABLE XVI

THE PROTHROMBIN TIME (ONE STAGE TECHNIQUE OF QUICK) AT VARYING INTERVALS AFTER WITHDRAWAL OF THE BLOOD

CONCENTRATION OF PLASMA (%)	PLASMA PROTHROMBIN TIME (SECONDS)				
	HOURS AFTER WITHDRAWAL OF BLOOD				
	½ HR.	1 HR.	2 HR.	4 HR.	8 HR.
100*	16	14	15	19	18
10*	25	23	25	28	38

*Both specimens kept at room temperature.

It appears that in the undiluted specimen there is no prolongation of the plasma prothrombin time during the first 4 to 8 hours, while a moderate prolongation is noted when the plasma is diluted to 10 per cent.

In order to obtain definite information as to the actual reduction in a series of investigations the two-stage titration technique was employed. The series of dilutions of the serum was divided into two parts, one being left on the laboratory desk throughout and the other being placed in the refrigerator. A characteristic series of observations is given in Table XVII.

TABLE XVII

THE LATENT PERIOD (TWO STAGE TECHNIQUE OF WARNER ET AL.) AT VARYING INTERVALS AFTER WITHDRAWAL OF THE BLOOD

SPECIMEN—10 PER CENT DILUTION	LATENT PERIOD (SECONDS)		
	HOURS AFTER WITHDRAWAL OF BLOOD		
	½ HR.	2 HR.	4 HR.
Specimen kept in refrigerator	15	14	16
Specimen kept at room temperature	14	20	27

The results clearly indicate a significant reduction in quantities of prothrombin upon leaving the specimen exposed to the light and temperature of the laboratory.

By referring again to Fig 52, it will be understood that the regular determination of the prothrombin time in this connection is of less significance, as it cannot record reductions in quantities of prothrombin less than about 60 per cent of the original normal quantity With the two-stage titration technique the setup is entirely different During the course of the titration one finally ends up with a sample containing one unit of prothrombin. Any reduction in this quantity will immediately be reflected through a prolongation of the latent period.

As emphasized by Warner and co-workers, it is extremely important to guard against deceptive results occurring on the basis of in vitro deterioration of prothrombin The blood must be withdrawn without any formation of even the smallest blood clot. After withdrawal I prefer to keep the specimen in the refrigerator during the various stages of the procedure prior to the actual performance of the titration

believe that practical applicability and dependability of a routine technique are obtainable primarily through the use of oxalated or citrated plasma and emulsions of thromboplastin sufficiently potent to prevent errors through possible variations of the concentration of this factor.

2. Titrations with varying concentrations of calcium chloride of specimens with varying concentrations of the anticoagulant solution. The basis of this technique is that the velocity of the reaction decreases with concentrations of calcium chloride above and below the range of the maximal coagulant effect of calcium chloride. Also, in this case it would be necessary to decide upon an arbitrarily chosen, fixed coagulation time to be reached by titration with various concentrations of calcium chloride, either below or above the range of its maximal coagulant effect. Specimens with decreased concentrations of prothrombin would require a relatively lower concentration of calcium chloride above, and a relatively higher concentration of calcium chloride below, the range of maximal coagulant effect of calcium as compared to the normal.

3. Recalcification of a series of specimens with constant concentration of calcium chloride, the samples of the series being obtained with increasing concentrations of the anticoagulant solution. This approach follows from the finding that the coagulation time of a given specimen recalcified with constant concentration of calcium chloride increases with the concentration of the anticoagulant solution (Fig. 49). By standardizing the technique upon a given coagulation time the deviation of the series from that of normal would be an expression of differences in the quantity of prothrombin. It will be obvious that this possibility, like the previous one, has no practical consequence.

4 Modified one-stage titration technique of Quick, employing increasing dilutions of the plasma, the titration aiming at an unknown dilution of the plasma which will give an arbitrarily chosen, fixed velocity (Figs. 52 and 54).

5. A procedure identical to the preceding one, but utilizing serum prepared for the regular two-stage technique instead of plasma (Fig. 54).

6. The two-stage technique of Warner and his associates

Evidence has been presented indicating that the latter three procedures may be employed with equal success in the quantitative estimation of prothrombin when strict attention is paid to essential technical details

7. Completing this survey it should finally be mentioned that quantitative estimation of prothrombin is possible by determination of the period of incubation necessary for complete conversion of that particular concentration of prothrombin (Fig. 56). This procedure would be most complicated and most unreliable.

In Vitro Reduction of Quantities of Prothrombin

For practical purposes it is important to ascertain whether or not changes in the quantities of prothrombin occur in the specimen after it is obtained.

An orientation as regards this possibility was sought by determinations of the plasma prothrombin time in the regular manner at different intervals after

determined by a stop watch. At the end of the period of incubation the fibrinogen is added in a quantity of 0.2 c c. Simultaneously the blind test is replaced in the apparatus by the absorption cell Regularly the film is permitted to run for one or two minutes; that is, sufficiently long to assure a complete recording of the process. It is to be understood that the film cannot be developed after each serial investigation. This is done at the end of the day's investigations During the two-stage procedures, however, it is necessary to ascertain when a latent period of 15 seconds is finally reached. This is done conveniently during the actual running of the test by observing the move-

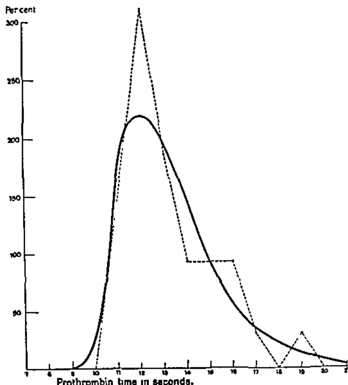


Fig 56 —The frequency distribution curve of the normal prothrombin time, based on observations of 32 adults

ments of the light reflected from the amperemeter mirror After the development of the film the reading of the recording according to the technique previously described is compared with that visually observed Both observations have checked very well so as to convince me that the photo-electric recording of the process is not actually necessary so long as the photo-electric arrangement permits a satisfactory visual observation of the kind indicated When I have regularly recorded all the observations, the photo-electric recording is done nevertheless in order to retain a permanent record of the entire process.

Standard Technique for Prothrombin Determinations

The technique for withdrawal of the blood for prothrombin determinations differs in no way from that described when investigations of the coagulability of blood plasma are to be performed. Immediately after the blood is drawn, the specimen is centrifuged at 2,000 to 3,000 revolutions per minute for 5 minutes and the supernatant plasma is pipetted off.

Determination of the Regular Plasma Prothrombin Time.—I have followed essentially the technique as described by Quick.

From the centrifuged plasma 0.3 c.c. is pipetted off into the specially designed absorption cell. To this is added 0.2 c.c. thromboplastic emulsion of 100 per cent. The absorption cell with its content is now placed in the water bath at 37° C. for 10 to 15 minutes. In the water bath is also placed the recalcifying solution of 0.3 per cent calcium chloride. When the reactants have reached the desired temperature, the test is done as follows:

The rotation of the film in the photelgraph is started. A blind test is introduced into the socket for the absorption cell. The aim of this arrangement is to obtain a horizontal line on the film which can indicate the constancy of the illumination and further indicate the zero point of the reaction when this horizontal line is interrupted upon removing the blind test. At the moment of addition of 0.4 c.c. calcium chloride the blind test is replaced by the absorption cell containing the system under investigation. Adjustment of the resistances to the fixed arbitrarily chosen value of illumination is undertaken *after* recalcification. The progression of the reaction is observed through the window of the apparatus. The arrest of the movements of the reflected light from the amperemeter mirror serves as indicator of the point after which the running of the film may be safely arrested without running the risk of obtaining an incomplete recording. The recording is read, according to the technique previously described, after development of the film.

Estimation of Prothrombin According to the Two-Stage Technique.—The technique has not differed essentially in any point from that described by Warner and his associates A convenient volume of the centrifuged plasma is coagulated by one-tenth its volume of a fresh thrombin solution as represented by serum expressed from a freshly coagulated specimen After complete inactivation of thrombin present, a series of dilutions of the serum is arranged, using a physiologic saline solution as diluent

From each sample of the series 0 3 c.c is pipetted into the individual absorption cells To this is added 0 2 c.c. of a 100 per cent thromboplastic emulsion. The whole series of cells is placed in the water bath at 37° C. together with the recalcifying solution of 0.3 per cent calcium chloride, and the fibrinogen solution prepared according to the same technique employed by Warner and co-workers. After the various reactants have attained the constant temperature, the film is started with the blind test in place, as described above.

The actual conversion of prothrombin is accomplished through the addition of 0 4 c.c. calcium chloride. The duration of the incubation period is

TABLE XIX

SERIAL OBSERVATIONS OF THE PLASMA PROTHROMBIN TIME IN THE SAME SPECIMEN (ONE STAGE TECHNIQUE)

CASE	PROTHROMBIN TIME (SECONDS) OBSERVATION NO.												ARITHMETIC AVERAGE	STANDARD DEVIATION
	1	2	3	4	5	6	7	8	9	10	11	12		
33	17	13	15	12	15	16	14	14	14	16	15	15	14.7	1.4
34	14	20	20	15	16	16	16	14	18	18	17	—	16.7	2.0
35	30	35	33	39	36	35	37	39	34	35	40	—	35.7	2.8
36	96	86	80	80	106	90	96	86	94	92	88	—	90.4	7.3

Summary

A survey has been presented of previous investigations concerning the coagulation of blood through the so-called tissue extracts.

There are two kinds of tissue extracts: (1) The thermolabile aqueous or saline extracts of Morawitz exhibit a very high coagulant potency. The active constituent is possibly a protein compound of a derivative of a phospholipoid. (2) The thermostabile alcoholic extracts of Schmidt are less potent. Their coagulant activity is considered to be exerted mainly through the phospholipoid, cephalin.

In the present work the tissue extracts have been considered under the neutral term thromboplastic material, thromboplastic emulsions, or thromboplastin.

A brief account is given of Howell's works concerning a qualitative and quantitative study of prothrombin through the employment of thromboplastic material, this work leading to the quantitative investigations of prothrombin by Roderick in "sweet clover disease" of cattle and further to clinical methods for quantitative estimation of prothrombin by the *one-stage technique* of Quick or the two-stage technique of Warner and his associates.

The photelgraph has lent itself readily to photo-electric recording of the coagulation of recalcified oxalated or citrated plasma to which has been added excess of thromboplastin, as well as to recordings of the latent period of thrombin activity as an essential part of the two-stage technique of Warner and associates.

The thromboplastic emulsions employed in the present study were prepared in controlled quantity and potency from powdered material obtained by acetone extractions of macerated rabbits' brain

An interpretation of the automatic photo-electric tracings of coagulation of recalcified plasma containing excess of thromboplastin revealed that *the first formation of fibrin occurred at point F. Geometrically point C is well defined in fast-reacting systems. It indicates only relative completion of fibrin formation, as minute quantities of fibrin are being formed for a considerable period beyond this point. In slower reactions point C is very poorly defined due to the gradual formation of fibrin.* It is therefore evident that with the present reaction the photo-electric tracings do not naturally divide the process of coagulation of this type into several stages as was the case in plain recalcifi-

On the basis of the dilution giving a latent period of 15 seconds, that is, containing one unit of prothrombin, the concentration of prothrombin in units per cubic centimeter of genuine plasma can be reached by computation.

The Normal Prothrombin Time

With the standard technique as described above I have determined the prothrombin time in thirty-two normal individuals (Table XVIII).

TABLE XVIII

THE PLASMA PROTHROMBIN TIME IN SECONDS IN 32 NORMAL ADULTS (ONE STAGE TECHNIQUE)

CASE	PROTHROMBIN TIME (SECONDS)
1	13
2	15
3	13
4	15
5	12
6	12
7	12
8	11
9	12
10	11
11	12
12	12
13	14
14	12
15	16
16	16
17	13
18	12
19	19
20	11
21	13
22	15
23	17
24	11
25	12
26	14
27	11
28	12
29	16
30	13
31	13
32	14

Statistical computation of this series gave an average prothrombin time of the normal of 13 3 ± 0.35 seconds.

The computed frequency distribution curve is given in Fig 56. The asymmetric type of curve is understood when considering that the free distribution is limited toward lower values.

In order to investigate the experimental error of the individual determination of the prothrombin time, I have in five cases performed a serial determination of the prothrombin time on the same specimen (Table XIX). It is to be noted, as was the case with the coagulation time of the plainly recalcified plasma, that there is an inverse relation between the probable error and the velocity of the reaction.

system intended for measuring the velocity of coagulation it is therefore essential to standardize the technique at a constant temperature

In recalcified plasma containing excess of thromboplastin *the period of prothrombin conversion in human beings covers about three-fourths of the entire first period of the reaction;* that is, from the zero point of reaction to the first formation of fibrin. *The actual duration of the period of prothrombin conversion under standardized conditions depends upon the actual concentration of prothrombin.*

By standardizing the reacting system upon constant concentrations of thromboplastin and active calcium ions, the varying velocity of the reaction constitutes a relative expression of the quantity of prothrombin present This is the basis of the one-stage technique for quantitative estimation of prothrombin as described by Quick.

Concerning the standardization of the technique it has been stressed that it is essential to operate with a potent emulsion of thromboplastin, far in excess of its critical point of activity at which concentration or potency even small volumetric variations in the thromboplastic emulsion will greatly influence the velocity of the process.

Emphasis has further been put on the maintenance of a constant concentration of active calcium ions. This will necessarily have to include the maintenance of a constant concentration of an anticoagulant solution of a given concentration per volume unit of genuine plasma. Consequently due consideration has to be paid to the varying hematocrit values in each individual case, in order to be able to determine in each case the changing volume of the anticoagulant solution of a given concentration which is to be added to a constant volume of whole blood By this procedure only is it possible to satisfy partially the requirements of a constant concentration of active calcium ions Variations in concentrations of the inherent electrolytes of the blood are naturally not accounted for by this procedure When due reservations are made, it is suggested that the varying hematocrit values may be disregarded when the procedure is intended mainly for orientation about marked deficiencies in concentrations of prothrombin.

A graph has been presented correlating the prothrombin time with varying concentrations of prothrombin. As plainly indicated by this graph, its very form proves the inability of the regular prothrombin time of Quick to indicate anything more than a rough orientation as to variations in quantities of prothrombin It further indicates the fallacy of estimating percentages of prothrombin in relation to the normal on the percentage basis frequently encountered in the present literature *If determinations of the prothrombin time are retained as the relative expression of quantities of prothrombin, it is suggested that they be expressed in number of seconds and not in percentages*

A survey has been presented of various theoretic and practical possibilities for quantitative estimation of prothrombin

With the above-mentioned reservations, the determination of the prothrombin time according to the unmodified technique of Quick may be ad-

cation of plasma. The first stage of the process, from the zero point of the reaction to the first formation of fibrin, is indicated clearly irrespective of the velocity of the process.

The tracings between points F and C, regardless of the velocity of the reaction, exhibit a maximal velocity of fibrin formation at the very onset of the second stage of the process. This is interpreted as indicative of *the presence of a relatively maximal concentration of the two reactants, thrombin and fibrinogen, at the onset of the second stage of the process.* This is considered as substantiating the concept of Schmidt that tissue extracts exert their coagulant activity through the conversion of prothrombin to thrombin.

By decreasing the concentrations of thromboplastin added to recalcified plasma it is plainly shown that the photo-electric tracings in their geometric appearance are approaching that of plainly recalcified plasma. This is taken to indicate that under otherwise equal conditions the difference between the two types of reactions as indicated by the photo-electric tracings is only apparent, and results from an increased velocity of the reaction as produced through the higher concentrations of thromboplastin *In every other respect the kinetics of the two types of reactions are essentially identical.*

The first stage of the process from the zero point of reaction to the first formation of fibrin (at point *F*) has been retained as the quantitative expression of the velocity of the process (the prothrombin time of the reaction).

This first period concerns the conversion of the prothrombin to thrombin plus the latent period of the first quantity of thrombin formed.

The period of thrombin formation in this reaction, as in plainly recalcified plasma, continues during the second stage of the process In contrast to the latter, however, the present reaction exhibits evidence of a physical inactivation of thrombin. This inactivation is governed by the velocity of the reaction. It appears to be a direct result of the rapid initial transformation of the specimen from a sol to a gel. This physical inactivation of thrombin decreases with the velocity of the fibrin formation until it reaches a point where it is able to exercise a complete transformation of fibrinogen to fibrin without interference from the latter. At this velocity the photo-electric tracing of the reaction is identical in type to that of the plain recalcification of the plasma. It is important to note, as also previously pointed out, that this is the type of tracing also characteristic for the spontaneous coagulation of genuine plasma. From a teleologic point of view it appears consequently of basic importance, in order to obtain complete transformation of fibrinogen, that the blood does not contain a too high concentration of thromboplastin

Quantitative investigations of the reaction have revealed that *the rate of the conversion of prothrombin to thrombin is determined by the following factors:* (1) *the concentration of prothrombin;* (2) *the concentration of thromboplastin;* (3) *the concentration of the active calcium ions.*

The conversion of prothrombin is further influenced by *the temperature.* The same also holds true for the latent period of thrombin activity. In any

11. Zak, Emil: Studien zur Blutgerinnungslehre, Arch f. exper. Path. u. Pharmakol. 70: 27-54, 1912.
12. Zak, Emil: Studien zur Blutgerinnungslehre, Ibid. 74: 1-13, 1913.
13. Bordet, J., and Delange, L.: Betrachtungen uber die Rolle der Lipoide bei der Blutgerinnung, Ibid. 71: 293-295, 1913.
14. Bordet, J., and Delange, L.: Quoted by Wöhlisch, Edgar: Die Physiologie und Pathologie der Blutgerinnung, Ergebn. d. Physiol 28: 443 624, 1929
15. Howell, W. H.: The Nature and Action of the Thromboplastic (Zymoplastic) Substance of the Tissues, Am. J. Physiol. 31: 1-21, 1912.
16. Mellanby, John: The Coagulation of Blood, J Physiol. 38. 28-112, 1908.
17. Ferguson, J. H.: An Experimental Analysis of Coagulant Activation, Am. J. Physiol. 117: 587-595, 1936
18. Loeb, Leo, Fleisher, M. S, and Tuttle, Lucius: The Interaction Between Blood Serum and Tissue Extract in the Coagulation of the Blood. I. The Combined Action of Serum and Tissue Extract on Fluoride, Hirudin and Peptone Plasma; the Effect of Heating on the Serum, J. Biol. Chem. 51: 461-483, 1922.
19 Loeb, Leo, Fleisher, M S, and Tuttle, Lucius: The Interaction Between Blood Serum and Tissue Extract in the Coagulation of the Blood. II. A Comparison Between the Effects of the Stroma of Erythrocytes and of Tissue Extracts, Unheated and Heated, on the Coagulation of the Blood, and on the Mechanism of the Interaction of These Substances With Blood Serum, Ibid. 51: 485-506, 1922
20. Howell, W. H.: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int. Med. 13: 76 95, 1914.
21 Howell, W. H., and Cekada, E. B: The Cause of the Delayed Clotting of Hemophilic Blood, Am. J. Physiol. 78: 500 511, 1926.
22. Roderick, L. M: A Problem in the Coagulation of the Blood; "Sweet Clover Disease of Cattle," Ibid. 96: 413-425, 1931.
23. Quick, A. J.: On Various Properties of Thromboplastin (Aqueous Tissue Extracts), Ibid 114: 282 296, 1936
24. Quick, A. J., Stanley-Brown, Margaret, and Bancroft, F. W.: A Study of the Coagulation Defect in Hemophilia and in Jaundice, Am. J. M. Sc. 190: 501-511, 1935
25 Warner, E D, Brinkhous, K. M., and Smith, H. P.: A Quantitative Study on Blood Clotting: Prothrombin Fluctuations Under Experimental Conditions, Am. J. Physiol. 114. 667-675, 1936.
26. Dam, H., and Glavind, J.: Vitamin K i den menneskelige patologi, Ugesk. f. laeger. 100. 248 250, 1938
27. Warner, E. D, Brinkhous, K. M., and Smith, H P. Plasma Prothrombin Levels in Various Vertebrates, Am. J. Physiol 125 296 300, 1939.
28 Owen, C A., Hoffman, G R, Ziffren, S E, and Smith, H P.: Blood Coagulation During Infancy, Proc Soc Exper. Biol & Med. 41 181-185, 1939.
29 Klinke, Karl Kolloidchemie der Gerinnung In Lichtwitz, Leopold, Liesegang, R. E. and Spiro, Karl Medizinische Kolloidlehre. Physiologie, Pathologie und Therapie in Kolloidchemischer Betrachtung Dresden, 1934, Theodor Steinkopff, pp 416-434.
30. Landsberg Quoted by Klinke, Karl[29]

vantageously used for clinical orientation concerning marked deficiencies of prothrombin. When actual study of the concentration of prothrombin is intended, other procedures must be employed.

There is principally no actual difference in the dependability of the one-stage and the two-stage techniques of quantitative prothrombin determination when retaining the technique of titration for both; that is, dilutions of the specimen to a concentration exactly exhibiting a conveniently chosen, fixed velocity of the *reaction. For clinical and experimental purposes we are consequently* in the possession of three different methods of equal quantitative significance. These are: (1) The two-stage titration technique of Warner and co-workers; (2) the one-stage titration technique employing oxalated or citrated plasma (the titration of a fixed "plasma prothrombin time"); (3) the one-stage titration technique employing serum as regularly prepared for the two-stage technique (the titration of a fixed "serum prothrombin time").

It is to be pointed out that all three procedures require the use of a specially prepared solution of fibrinogen. As readily understood, extensive dilution of the plasma will result in dilutions of the plasma fibrinogen too extensive to yield accurate end points without applying a similar technique as used in the present work

Studies of the in vitro changes of the blood specimens revealed an appreciable depreciation of prothrombin within two hours after withdrawal of the blood, when the specimens were exposed to the light and temperature of the laboratory. For this reason the specimens ought to be placed in the refrigerator immediately after withdrawal. Even extensive centrifugation of the specimens is of no consequence for the quantitative results.

A description is given of the standard technique for quantitative estimation of prothrombin.

The normal prothrombin time in human beings was found to be 13 3 ± 0 35 seconds.

The experimental error of a single observation was found to be increasing with the actual prothrombin time

References

1. Pickering, J. W. The Blood Plasma in Health and Disease, New York, 1928, The Macmillan Co., 247 pages
2. Buchanan, Andrew: On the Coagulation of the Blood and Other Fibriniferous Liquids, J. Physiol. 2: 158-163, 1879-1880
3. Halliburton, W. D.· On the Nature of Fibrin Ferment, Ibid. 9: 229-286, 1888.
4. Schmidt, Alexander: Quoted by Wöhlisch, Edgar· Die Physiologie und Pathologie der Blutgerinnung, Ergebn d Physiol. 28 443-624, 1929
5. Wooldridge, L C.. Further Observations on the Coagulation of the Blood, J. Physiol. 4· 226 230, 1882 1883.
6. Wooldridge, L. C. On the Coagulation of the Blood, Ibid. 4: 367-369, 1882-1883.
7. Halliburton, W. D.· The Proteids of Kidney and Liver Cells, Ibid. 13: 806-846, 1892
8. Morawitz, P.· Die Chemie der Blutgerinnung, Ergebn. d. Physiol. 4: 307-422, 1905
9. Fuld, E., and Spiro Quoted by Pickering, J. W.[1]
10. Freund, E.. Quoted by Wohlisch, Edgar: Die Physiologie und Pathologie der Blutgerinnung, Ergebn. d. Physiol 28: 443-624, 1929.

seems essential to further comment. Without entering into a controversy regarding the monophyletic and polyphyletic theories of blood formation, it is agreed that the blood vessels during embryonic life are formed from the same area as the formed elements; namely, from blood islets in the area vasculosa of the syncytial mesoderm. *On this basis it seems logical to assume a certain* biologic relationship between the endothelium and the reticuloendothelial elements. In other words, ample evidence indicates that under certain pathologic conditions this biologic relationship results in significant changes both in the corpuscular elements and in the endothelium. Consequently, the classification of these particular conditions seems evident. As hemorrhagia per *diapedesin* is the most prevalent type of hemorrhage among the clinical conditions to be considered presently, it is clear that there are grounds for controversy.

Histology of Hemorrhages.—By microscopic observations of subpericardial hemorrhages Ribbert (1915) observed that these hemorrhages or petechiae consisted mainly of erythrocytes with an almost complete absence of leucocytes The *greatest number of leucocytes* was found *in the region surrounding* the vascular lesion, while a smaller number was found in the intravascular thrombus In animal experiments Ribbert, by the prick of a needle, made small openings into a small artery or a vein After killing the animals by means of anesthesia, after varying intervals, the site of hemorrhage was excised and *sections were made after freezing. As in human beings*, there was retention of leucocytes in the region surrounding the vascular lesion, the leucocytes together with the platelets and strands of fibrin forming the vascular thrombus. During the formation of the extravascular thrombus, erythrocytes continue to flow through, thereby enlarging the hemorrhage, which contains *fewer leucocytes at its periphery*

Conditions are different in the presence of an aneurysm where the blood is forced out of the vascular lumen with great force All the corpuscular elements of the blood are carried out *together into the* tissues. The precipitation of fibrin, platelets, and leucocytes in the periphery forms the sac which by additional precipitation increases in thickness

By producing small lesions in the arterial intima without any extravasation of blood, Ribbert observed the formation of a platelet thrombus covering the intimal lesion *This first precipitation of platelets is enlarged by the additional* agglutinating platelets supplied by the circulating blood

The Arrest of Hemorrhage—When hemorrhage occurs, certain phenomena have been found to take place Viewed as a whole these phenomena appear to present a defensive mechanism against hemorrhages.

1 When hemorrhage ensues after external trauma, immediately retraction and contraction of the wound surface occurs because of severance of elastic tissues After cutting the capillaries of a frog, Herzog observed microscopically a spastic contraction or gluing together of both *ends of the capillary* wall, which for a time prevented blood from escaping In the adjacent parts

PART III

CHAPTER IX

CLASSIFICATION OF HEMORRHAGIC DISEASES

Introduction

In the present part of the work it is my intention to present the result of studies on the coagulability of the blood in various clinical conditions that are characterized by hemorrhagic manifestations. Thereby an impression may be gained of the clinical applicability of the tests considered in the first part of this work, as well as the clinical value of investigations of the coagulability of the blood in general in these conditions. Where such is warranted, points of consequence for consideration of the pathogenesis of hemorrhage will likewise be presented. Clinical problems of essential importance are to be considered only when more or less directly related to the investigations of the coagulability of the blood

Anyone occupied with problems of the varied conditions revealed by hemorrhagic manifestations naturally gropes for some line of thought concerning the process through which hemorrhage is produced in these conditions Peculiarly enough, this activity seems inevitably to lead to rearrangement of the classification of hemorrhagic diseases, hence the abundance of classifications in the medical literature. This ought to be sufficient warning to me to preserve my classification for my own use only. When, after due consideration, this objection nevertheless is over-ruled, it is done for one reason; namely, it is expected that a classification of the hemorrhagic manifestations may clearly express better than anything else my conception of the present status of essential problems related to these conditions and simultaneously indicate possibilities for further progress Before reaching this point it is necessary to present some of the knowledge of direct significance for an understanding of the mechanism of hemorrhage.

The Pathologic Changes of Hemorrhages.—Pathologists generally consider the subsequent two types of hemorrhage·

1. *Hemorrhagia Per Rhexin.*—The blood escapes from the vascular system through a gross lesion in the vascular wall. This lesion may be caused by external trauma or by an internal factor (without or with pathologic changes in the vascular wall).

2. *Hemorrhagia Per Diapedesin.*—The blood escapes the vascular system without any gross lesion of the vascular wall but, as is assumed, between the endothelial cells. If this latter type of hemorrhage is considered, one point

intravascular pressure and thus arrest the hemorrhage. Viewed from this mechanical angle typical localizations of hemorrhagic manifestations are thus readily explained.

6. When the hemorrhage is excessive, endangering the life of the patient, the parallel drop in the blood pressure to some extent may counteract, if not prevent, further loss of blood.

As intimated, the various factors active in the course of hemorrhage give the impression of constituting a finely graded antihemorrhagic mechanism. The tendency to view any apparently coordinated mechanism from a teleologic point may harbor many fallacies At any rate it seems beyond question that, deprived of the mechanism here considered, we should all have to move in our daily life very much like hemophiliacs, knowing that small traumas might result in serious hemorrhage. The maintenance of this protecting mechanism must be considered of vital importance. Its maintenance is ensured through the correlated activity of a series of organs. Essential among these are the bone marrow and the entire reticuloendothelial system which control the production and life cycle of the corpuscular elements of the blood; the liver (and the bone marrow?) through the formation of fibrinogen and prothrombin; the tissues as the main source of tissue coagulants; the parathyroid glands as controlling the calcium metabolism; the gastrointestinal tract as the source as well as the conveyer of certain vitamins of basic importance for the production of prothrombin; and the intact nervous system through its reflex regulation of the vascular tree. Considering finally the most varied and graded processes of direct consequence for the maintenance of normal osmotic pressure, one may have obtained a picture, admittedly incomplete, nevertheless clear enough, of the magnitude of the problem concerning hemorrhages and the arrest of hemorrhages

The Pathogenesis of Hemorrhages.—We have no definite knowledge concerning the finer mechanism that causes hemorrhages in various clinical conditions

We know there is a group of conditions associated with hemorrhagic symptoms and in which the blood differs in no way from that of normal conditions. In such instances the hemorrhages are generally considered to be purely vascular

There are other conditions in which definite hematologic changes are known to occur In the following chapters we shall consider four different clinical conditions in which deficiencies in blood coagulability or in the number of platelets are outstanding pathologic features. This does not exclude the possibility that vascular pathologic changes play an important part in such conditions. If they do, we are still at a loss to explain whether the vascular and hematologic changes may occur on the same etiologic basis or whether vascular changes may occur secondarily after primary changes in the blood.

During the following chapters we shall observe a third factor. Whether or not there is a primary vascular deficiency, the localization of the hemor-

of the severed capillaries a stasis of blood was noted. Neighboring capillaries were found to be in a state of dilatation. Herzog considered this a substantiation of the concept of Stegemann[1] of an autonomous sidetracking of the blood stream (*"Selbststeuerung des Kreislaufes"*). The blood takes the route of the least resistance away from the severed, congested capillaries into the surrounding dilated parts of the vascular tree. This is also the concept of Magnus,[2] who found that post-traumatic spasm lasted for several minutes. An exception to this rule was found if local hyperemia was present or if the nervous reflex mechanism had been interrupted by the local application of cocaine.

2. When the vascular lesion is very small, a mechanical closure is attempted by the platelets which, due to their agglutinating and adhesive function, will stick to the orifices of the broken capillaries and arterioles, thus plugging them. The platelets that escape through the opening disintegrate rapidly under liberation of thromboplastin, thereby exerting a further antihemorrhagic effect through the coagulating blood. The assumption that the disintegrating platelets by their disintegration liberate a pressor substance directly effecting constriction of the capillaries must be considered problematic.

3. Hand in hand with the mechanical closure of the lesion by platelet plugging goes formation of a thrombus through the coagulating blood In larger lesions this mechanism must be considered of significance for the successful arrest of the hemorrhage All the factors necessary for the coagulation of blood are normally found to be present in the blood itself (prothrombin, thromboplastin, calcium, fibrinogen). During traumatic hemorrhages these factors are supplemented by tissue coagulants which in reality means an increase in the concentration of available thromboplastin The tissues, as well as the above-mentioned mechanical factor, consequently exert a most significant chemical action through the liberated thromboplastin Considering this double antihemorrhagic role of the tissues, the prevalence of hemorrhages from mucous membranes is understood on the basis of the bleeding occurring not only into an open space but into one lacking the coagulant effect of abundant tissue coagulants.

4. During heavy loss of blood an automatic regulation of the maintenance of a sufficient blood volume is effected through increased diffusion of tissue juices into the blood stream As a consequence such patients rapidly appear dehydrated It is to be expected that a certain additional quantity of tissue coagulant thereby is brought into the circulating blood, in its turn increasing the coagulability of the blood. This exchange no doubt is important also for the maintenance of normal coagulability under normal conditions It may further explain the bizarre findings of the normal coagulability of the blood that is sometimes present in cases in which there occurs a marked degree of primary or secondary thrombocytopenia with purpuric manifestations.

5. When bleeding occurs into closed tissues, the internal pressure of the hematoma resulting from the surrounding tissues may approach or equal the

clearly indicated the double role played by the platelets in the arrest of hemorrhage; for this reason I believe cognizance of this fact should be observed in the classification so far as it conforms with observations concerning hemophilia and essential thrombocytopenic purpura, to be dealt with later.

TABLE XX

CLASSIFICATION OF HEMORRHAGIC MANIFESTATIONS

A. *Hemorrhagic manifestations of nonhematologic origin*	1. Definitely traumatic and surgical 2. Manifestations on purely mechanical basis: (a) thrombophlebitis, (b) cardiac insufficiency 3. Bacterial emboli: (a) variola, (b) meningococcus meningitis 4. Purpura in infectious diseases 5. Toxic purpura 6. Schönlein Henoch's purpura 7. Hereditary telangiectasia (Osler) 8. Scurvy	
B. *Hemorrhagic manifestations of hematologic origin*	Reduction in the number of platelets:	1. Essential thrombocytopenic purpura 2. Aplastic anemia 3. Acute leucemia 4. Pernicious anemia 5. Toxic thrombocytopenia: (a) arsenic, (b) benzol, (c) salvarsan, (d) radium, (e) x rays, (f) thorotrast, (g) snake venom 6. Infectious thrombocytopenia: (a) typhus, (b) sepsis, (c) variola, (d) malaria, (e) kala azar, (f) endocarditis lenta 7. Thrombocytopenia with splenomegaly: (a) Banti's disease, (b) Gaucher's disease
	Functional impairment of platelets:	1 Thrombasthenia (Glanzmann) 2 Hemophilia (?)
	Quantitative deficiency of: Fibrinogen:	1 Fibrinopenia
	Thromboplastin:	1 Hemophilia 2 All conditions with thrombocytopenia
	Prothrombin:	1 Hypothrombinemia hemorrhagica neonatorum 2. Hypoprothrombinemia in cases of disease of the gall bladder and bile ducts, liver and pancreas; sprue, other intestinal disorders

References

1 Stegemann, Hermann Zur Frage der Blutstillung in der Chirurgie, insbesondere des spontanen Blutungsstillstandes, Beitr. z. klin Chir. 127, 657-673, 1922.

2 Magnus: Quoted by Stegemann, Hermann.[1]

rhagic manifestations indicates the great influence played by trauma as the precipitating factor. Thus is created a vascular deficiency which, in the presence of hematologic changes, immediately or subsequently, allows an extravascular accumulation of blood. When such an extravascular accumulation of blood is developing, it is readily understood that the graded mechanism considered above as active in arresting this development will be deficient because of essential hematologic abnormalities. In other words, *regardless of our lack of knowledge concerning the detailed mechanism leading to hemorrhagic manifestations in certain clinical conditions, the various hematologic abnormalities reveal why the hemorrhages are not normally checked when they are developing*

Admittedly, information on which to base an outline of the physiopathology of hemorrhages is meager. In fact, such an outline cannot be presented Looking at the problem from a purely clinical point of view, the outline presented above nevertheless indicates that hemorrhagic manifestations result from a series of factors forming a vicious circle in which certain hematologic factors are an essential link. This point affords the possibility of prevention or treatment of hemorrhagic manifestations through correction of the hematologic deficiency where such is present, and where such correction is obtainable with our present knowledge. Amplification of this point is to be presented in the subsequent clinical exposition

Classification of Hemorrhagic Manifestations.—The previous comment naturally leads to a consideration of the classification of the hemorrhagic manifestations.

A classification on an etiologic basis would be very desirable, only this cannot be done satisfactorily at present Neither can it be based on pathologicoanatomic data The various classifications used today are commonly on a purely clinical basis and as such may serve definite practical purposes

In suggesting a different type of classification, I am aware that it may not clarify the situation at all During the present work I have found it convenient to distinguish between conditions about which nothing definite is known and those concerning which some essential information is available. It so happens that the latter conditions include those in which essential hematologic changes are present; the other conditions are those generally considered of purely vascular origin It is not intended to present a complete classification of hemorrhagic manifestations but to construct a skeleton of a simple working hypothesis that emphasizes the functional importance of the hematologic factors while reserving judgment as to the possibly essential importance of the vascular factors (Table XX). It is further to be stressed that it is not a classification of hemorrhagic diseases but of hemorrhagic manifestations One finds, therefore, somewhat unexpectedly I admit, a condition such as thrombophlebitis included in the classification This is done because it represents a good example of one of the various ways by which hemorrhagic manifestations may be created. For the same reason hemophilia and conditions associated with thrombocytopenia have been listed as falling under two different headings, as may be seen in the table. The previous discussion has

by improved technique, found that prothrombin was present in normal quantities in hemophilia. Many previous reports had indicated the presence of large quantities of thrombin in hemophilic serum, thus making the quantitative deficiency of prothrombin unreasonable.[6,10] Howell and Cekada's findings have subsequently been substantiated by several workers.

Addis[3] proposed that, even though prothrombin is present in normal amounts, an abnormality of coagulation in hemophilia may be created through a *qualitative deficiency* of prothrombin, making it less convertible into thrombin, thus explaining the slow appearance of thrombin. Similar views have been explained by several subsequent workers,[15,18,20,21] although others have maintained that a qualitative deficiency does not exist.[14]

5. **Thromboplastin.**—Sahli,[3,4] who must be credited with being the first to undertake a more controlled analysis of the hematologic defect in hemophilia, conceived that the blood of hemophiliacs lacked in thrombokinase. "... *muss also angenommen werden dass die zelligen Elemente des haemophilen Blutes gegenuber denen des normalen Blutes einen chemischen Defekt darbieten indem sie entweder weniger der wirksamen substanzen (Thrombokinase) enthalten oder dieselbe weniger leicht abgeben.*" Morawitz and Lossen[10] concurred in this view and added that the defect most likely was of hereditary nature, comprising not only the corpuscular elements of the blood but also the extravascular tissues (*"Ererbte Abartung des Protoplasmas"*). Schloessmann[22] expressed an opinion similar to Sahli's. Subsequent reports[23,24] indicated that tissue extracts obtained at post-mortem examination of patients with hemophilia did not indicate any deficient potency as compared to that of normal subjects.

6. **The Platelets of Hemophiliacs.**—Great controversy has raged concerning the role of the platelets in hemophilia, the divergent views obviously in part due to disagreement of the role of the platelets for coagulation in general, partly to disagreement as to whether these cells contain thromboplastin or prothrombin, and not least, to various concepts of what constitutes a dependable and controllable technique when these corpuscular elements are transferred from their physiologic environment in genuine plasma. Mention has already been made of Sahli's concept. His views were partly substantiated by Fonio,[25,26] who stated that, whereas the hemophilic platelets did not exhibit any quantitative defect so far as their content of thrombozyme was concerned (thrombokinase, thromboplastin), the platelets nevertheless exhibited a qualitative defect in liberation of thrombozyme. Minot and Lee's[12] extensive investigations on this point indicated to these workers that the platelets in hemophilia are characterized by a hereditary defect, making them only slowly available for coagulation. Of particular interest in this connection is the observation by Opitz and Zweig[27,28] that the coagulability of oxalated plasma is increased by standing for several hours in contact with the cellular elements of the blood, while such a change in coagulability could not be detected in the absence of the platelets. It seems rather embarrassing that the blood in a hereditary disease of this grave nature and so difficult to influence beneficially in vivo should correct itself in vitro merely upon standing. The observation

CHAPTER X

HEMOPHILIA

Anyone interested enough to try a courageous dive into the literature on hemophilia may have experienced the same bewilderment I did when I realized that, according to the reports, practically everything concerning the pathology of blood coagulation, including part of the pathology of vessels, has at one time or another been "proved" to explain hemophilia, only to be disproved *subsequently*. *The one indisputable fact which remains practically unchallenged is that "hemophilia is an inherited tendency in males to bleed";*[1] *further that this condition is associated with a pronounced hypocoagulability of the blood. It appears that only a physiologist would have a chance of finding his* way through this maze of conflicting results, and in order to be able to present a constructive critical review he would have to be an expert physiologist at that. For obvious reasons, therefore, the following review must be incomplete; it may, however, serve the purpose of presenting a certain background for some observations concerning hemophilia.

The following have been *considered the main important factors* in hemophilia:

1. **Fibrinogen.**—At present there is general agreement on the point that this protein is present in hemophilia in normal quantity[3-5] and reactivity.[5-7]

2. **Calcium.**—It has likewise been shown that calcium is not the defective factor.[5-9] So far as I have been able to ascertain there is no clinical condition of defective coagulating mechanism caused by calcium deficiency. The observations of *Hess*[8] *on a boy, aged 6 years, with a manifest hemorrhagic tendency* and prolonged coagulation time of recalcified plasma simultaneously with a moderate lowering of the blood calcium, at present can hardly satisfy the requirements for the postulation of a new, clinical entity, as was done by Hess, under the name hemophilia calcipriva. I have had the occasion to examine a few patients in whom post-thyroidectomy tetany developed and also those with sprue whose serum calcium value was between 6 and 8 mg. per 100 c.c. of blood, and I have not been able to find any changes in the coagulability of the blood of the patients on whom operation was performed nor any changes out of proportion to that caused by the deficiency of prothrombin in the patients with sprue.

3. **Inhibitory Factors.**—It has been fairly well established by now that the hemophilic blood contains neither an excess of antithrombin[3, 5, 10-12] nor antiprothrombin (heparin).[13]

4. **Prothrombin.**—In a first report (1914) Howell[9] advanced the hypothesis that hemophilia was due to a lack or *quantitative deficiency* of prothrombin, an opinion also expressed by others[6] In a later report Howell and Cekada,[14]

by improved technique, found that prothrombin was present in normal quantities in hemophilia. Many previous reports had indicated the presence of large quantities of thrombin in hemophilic serum, thus making the quantitative deficiency of prothrombin unreasonable.[5,10] Howell and Cekada's findings have subsequently been substantiated by several workers.

Addis[5] proposed that, even though prothrombin is present in normal amounts, an abnormality of coagulation in hemophilia may be created through a *qualitative deficiency* of prothrombin, making it less convertible into thrombin, thus explaining the slow appearance of thrombin. Similar views have been explained by several subsequent workers,[13,18,20,21] although others have maintained that a qualitative deficiency does not exist.[14]

5. **Thromboplastin.**—Sahli,[3,4] who must be credited with being the first to undertake a more controlled analysis of the hematologic defect in hemophilia, conceived that the blood of hemophiliacs lacked in thrombokinase. ". . *muss also angenommen werden dass die zelligen Elemente des haemophilen Blutes gegenüber denen des normalen Blutes einen chemischen Defekt darbieten indem sie entweder weniger der wirksamen substanzen (Thrombokinase) enthalten oder dieselbe weniger leicht abgeben.*" Morawitz and Lossen[10] concurred in this view and added that the defect most likely was of hereditary nature, comprising not only the corpuscular elements of the blood but also the extravascular tissues (*"Ererbte Abartung des Protoplasmas"*). Schloessmann[22] expressed an opinion similar to Sahli's Subsequent reports[23,24] indicated that tissue extracts obtained at post-mortem examination of patients with hemophilia did not indicate any deficient potency as compared to that of normal subjects

6 **The Platelets of Hemophiliacs.**—Great controversy has raged concerning the role of the platelets in hemophilia, the divergent views obviously in part due to disagreement of the role of the platelets for coagulation in general, partly to disagreement as to whether these cells contain thromboplastin or prothrombin, and not least, to various concepts of what constitutes a dependable and controllable technique when these corpuscular elements are transferred from their physiologic environment in genuine plasma Mention has already been made of Sahli's concept. His views were partly substantiated by Fonio,[25,26] who stated that, whereas the hemophilic platelets did not exhibit any quantitative defect so far as their content of thrombozyme was concerned (thrombokinase, thromboplastin), the platelets nevertheless exhibited a qualitative defect in liberation of thrombozyme. Minot and Lee's[12] extensive investigations on this point indicated to these workers that the platelets in hemophilia are characterized by a hereditary defect, making them only slowly available for coagulation Of particular interest in this connection is the observation by Opitz and Zweig[27,28] that the coagulability of oxalated plasma is increased by standing for several hours in contact with the cellular elements of the blood, while such a change in coagulability could not be detected in the absence of the platelets It seems rather embarrassing that the blood in a hereditary disease of this grave nature and so difficult to influence beneficially in vivo should correct itself in vitro merely upon standing. The observation

has been verified. To Opitz and his associate it indicated that thromboplastin was present in the platelets in normal quantity but, due to a peculiarity of the platelets, was made available to the plasma only slowly. Similar views have been expressed by other investigators.[7, 14, 15] Several workers,[5, 6, 17, 20, 21] however, have contended that the platelets in hemophilia are qualitatively and quantitatively equal to those of normal blood.

Finally, mention shall be made of some recent researches which through their investigative approach promise to be of consequence for the further development of the present problem.

The basis of the investigations of Patek and associates[17, 18] was the well-known observation that normal plasma contains an active principle which, upon addition in small quantities to hemophilic blood, produces normal coagulability. Their investigations aimed directly at isolation of this coagulant factor. It was found to be thermolabile, was stable at icebox temperature, and was active in very high dilutions. Filtered normal plasma treated by aqueous dilution and acidification with carbon dioxide to a pH of 5.9 or with acetic acid to a pH of 5 3 resulted in the precipitation of a substance which could be dried in vacuo and exhibited characteristics identical to that of the plasma factor. The active principle was found to be a globulin compound although proof was lacking that globulin was the active factor An identical treatment of hemophilic plasma resulted in a precipitate of less amount than that formed from the normal plasma, but exhibiting equal coagulant potency to that of the normal precipitate when investigated in comparable quantities. In isotonic saline solution the active compound, termed "globulin substance," exerted a coagulant effect both in vitro and in vivo and by the investigators it has been injected intravenously in cases of hemophilia, the beneficial effect on the coagulability of the blood lasting from 6 to 10 hours

Bendien and van Creveld[31] arrived at practically identical conclusions, but preferred to call the active compound "coagulation globulin." Both groups of investigators conceived the idea that prothrombin was the active part in the precipitate. Because of its quantitative deficiency in hemophilia they concluded that hemophilia is characterized by a specific alteration of the hemophilic prothrombin complex. Bendien and van Creveld excluded the possibility that the factor was thromboplastin by referring to the finding of Patek and co-workers that the active factor could be isolated from platelet-free plasma From only moderate impairment in coagulant activity of the filtered plasma such a conclusion is rather problematic It seems still questionable whether it is possible to obtain plasma free of thromboplastin by filtration

Howell,[2] in taking up the valuable lead offered by these studies, found that the active precipitate contains prothrombin as well as thromboplastin, an unavoidable combination with the technique employed by both groups of investigators. Significantly, Howell found that the active principle also could be precipitated from a prothrombin-free plasma, thus indicating thromboplastin as the active principle. While usually occurring in combination with fibrinogen, it was readily obtained from plasma rendered fibrinogen-free by

acidification, thus indicating that a terminology implying *the active principle* is a protein compound would be misleading. He further contended that this substance is essentially the same as that designated by Morawitz as the thermolabile thrombokinase Howell preferred the neutral term "plasma thromboplastin" in order to differentiate it from the similar or identical substance "tissue thromboplastin" obtained from the tissues The substance has been found to give a positive reaction for phosphorus and nitrogen and, according to Howell, may prove to contain a glycerophosphate group.

With these investigations of Howell, the ring is closed. We are again within the concept of hemophilia as suggested by Sahli. There is reason to believe that this basis will prove a great stimulus to further elucidation of the problem.

Personal Clinical Observations

During the last three years I have had occasion to investigate the blood of eight cases of hemophilia The investigative technique has been identical to that stated for the cases of essential thrombocytopenic purpura.

Results.—In Table XXI are listed some of the essential findings in the cases investigated. In all of them the bleeding time and clot retraction were normal and the blood platelets were within normal limits The tourniquet test did not produce any petechiae in these cases

Before considering the individual cases, it may be of interest to compare the present group of cases with that of thrombocytopenic purpura (Tables XXIII and XXIV) and of hemorrhagic conditions associated with jaundice (Tables XXIX-XXXII). On the basis of the most pronounced changes in the coagulability in each group it is then readily revealed that *the most pronounced abnormality of coagulation is found in cases of hemophilia, relatively less so in cases associated with diseases of the gall bladder, liver, and bile ducts and relatively least so in cases of hemorrhagic purpura* Within this quantitative relativity among the three groups there are, within each group, all gradations of the existing defect in the blood coagulability, thus in most instances excluding the diagnostic importance of the coagulability of the blood plasma without simultaneous determination of other hematologic factors like enumeration of the platelets, determination of the serum bilirubin, estimation of the number of erythrocytes and leucocytes with differential count of the latter These informations, with a carefully taken clinical history, form the basis for the clinical diagnosis In the groups with hemorrhagic diathesis the test for coagulability of the plasma takes its place among the other nonpathognomonic hematologic tests.

In this connection I shall touch upon the difficulties which may arise in trying to fit a definite clinical impression of hemophilia with certain hematologic reports or in failing to make a diagnosis on the questionable findings of changes in coagulability of the blood.

In three cases of the present series the coagulation time of the blood, according to the method of Lee and White, was considered within or bordering on the upper limit of normal (Cases 2, 3, and 7) It may be added that none

TABLE XXI

HEMATOLOGIC INVESTIGATIONS IN 8 CASES OF HEMOPHILIA*

CASE	AGE AND SEX	DATE	COAGULATION TIME OF RECALCIFIED PLASMA IN SECONDS	NUMBER OF PLATELETS PER CU. MM. OF BLOOD	COAGULATION TIME OF WHOLE BLOOD (METHOD OF LEE AND WHITE) IN MINUTES	FAMILY HISTORY	REMARKS
1	42 M	5/20/39	840 (15)†	335,000	105	Positive	From early childhood subcutaneous hematoma aft... trivial injuries; recurrent hemarthrosis, him slightly incapacitated; repeated epistaxis; excessive hemorrhage after tractions; no actual hemorrhage now
2	18 M	2/21/38	480	400,000	10	Positive	f patient in Case 3; a twin sister always ie brother died at age of 12 years from ige after fall on skis; repeated recur- arthrosis; limps slightly; no bleeding vestigation undertaken before tooth ex-
3	24 M	3/23/38	420	388,000	11	Positive	patient in Case 2; at 17 years of age eding after tooth extraction necessitat- 1 transfusion; leads active life; no now
4	16 M	2/ 1/38	780	245,000	17	Positive	atient in Case 5 (see also Cases 6 and ng tendency noted in early childhood; episodes severe epistaxis; repeated es into knee and ankle joints with rthritic sequelae; 2/2/38 admitted for ction, which was done after prelimi- fusion; only moderate hemorrhage; dmitted after ten days of constant owing tooth extraction; hemoglobin t; note moderate hypocoagulability nsfusion; in December, 1939, and 140, no actual hemorrhages
		2/ 2/38	Blood transf.				
		2/ 3/38	230				
		2/ 6/38	340				
		2/10/38	1,240				
		9/ 2/38	310	320,000	11		
		12/13/39	800 (17)				
		1/12/40	780 (13)	255,000			

*Bleeding time of Duke and retraction of the clot present in all cases

†Figures in parentheses, prothrombin time in seconds.

5	23 M	2/16/38 1/ 9/40	1,200 1,520	286,000 341,000	18 32	Positive	Brother of patient in Case 4; very severe bleeding after early phimosis operation; likewise when losing milk teeth; almost identical clinical picture to that of brother although less severe; is leading an active life, goes skiing and hiking
6	49 M	2/16/38	790	310,000	19	Positive	Maternal uncle of patients in Cases 4 and 5; severe bleeding on following occasions: after tooth extraction at age 13 years, after blow to kidney at age 21 years (hematuria for 8 days), after hemorrhoid operation at age 37 years; additional hemorrhages into joints, which are definitely arthritic at present and prevent activities such as boxing and skiing done in earlier days; no hemorrhage now
7	17 M	2/16/38	380	310,000	8	Positive	Nephew of patient in Case 6; cousin of patients in Cases 4 and 5; never in hospital because of hemorrhages; bruises easily; frequent ecchymosis after small injuries; on occasion has had small hemorrhages in joints, particularly ankle joints; no definite arthritic manifestations; is not as active as any of above relatives because "ankles particularly get tender and stiff when exercising too much"; no bleeding now
8	8 M	11/19/37 4/ 6/38 4/26/38 6/ 3/38 9/26/38 12/19/38 2/21/39	1 hr. 1 hr. 1,200 780 690 930 720 (13)	454,000 331,000 285,000	3 hr. 2½ hr. 40 min. 32 min.	Negative	When 2 years old very severe perineal bleeding after injury; at age 3 years two very severe episodes of epistaxis requiring blood transfusions; at ages of 5 and 6 years several attacks of bleedings into knee joint after trivial injuries; physical activity greatly restricted because of arthritic manifestations and necessity of avoiding injuries; for latter reason he is educated at home by governess

of these cases was admitted to the hospital. Case 2 was referred for investigation by a dentist who, upon noting that the boy limped slightly, had happened to inquire into his past and family history. Simultaneously the case of his brother was investigated. Case 7 was selected because the patient was closely related to three other patients (Cases 4, 5, and 6). None of the three patients had at the time any actual hemorrhage.

Similar reports[22, 33] of a normal coagulation time of the blood have been the subject of considerable debate. Howell[2] seriously questioned these reports and has pointed out that in his cases of true hemophilia the coagulation time of the blood frequently has run into hours. As emphasized in several places in this work, normal coagulation time of the blood must not be taken to indicate normal coagulability. The three cases considered here are examples of this In all three patients the coagulability of the blood plasma was definitely prolonged, although all three, according to their blood coagulability, would be listed as moderate cases of hemophilia. This seems to be well borne out by their clinical histories

Aside from the interest these three cases attract from a diagnostic viewpoint, *they raise another point of some consequence. From the present small series it* appears that all degrees of hemophilia exist, ranging from the very severe type to the very mild type. Apparently it is not a fact that a person either has or has not the recessive hemophilic factor. The difference between the various degrees of the pathologic condition, and between this and the normal, appears to be one of quantity rather than quality. This seems to be an important point to be considered, particularly when discussing the so-called sporadic cases of hemophilia, which according to extensive investigations seems to be acceptable.[28, 33]

Mention shall also be made of the observation in Case 4 on Sept. 2, 1938. Because bleeding was moderate after the first tooth extraction in February of the same year, the boy persuaded his dentist to extract another tooth without any preliminary investigation or preparation An upper left molar was removed, with only moderate immediate hemorrhage However, bleeding began late in the afternoon and continued during the night. The dentist was unable to check the bleeding and advised the boy to enter the hospital The boy was unwilling to return to the hospital but finally did so after having had constant oozing from the tooth socket for an additional nine days. At the time of admission he was somewhat weak and exhausted. His hemoglobin concentration was only 42 per cent The tooth socket was filled with blood clots around which blood was escaping in a slow stream The socket was cleaned out and packed with cotton dipped in sterile, fresh serum from a normal person, after which the boy was given a transfusion of blood Very slight oozing continued during the afternoon, but bleeding had ceased the next morning

Immediately before the transfusion of blood a specimen was obtained for study of the coagulability of the blood. The results are listed in Table XXI. The coagulation time of whole blood was normal, but a definite, although very moderate, hypocoagulability of the blood plasma was present

In one of the first four cases described by Sahli[4] in 1905 a normal coagulation time was observed during severe bleeding, with return of the coagulation time to abnormally long values after cessation of the bleeding. Similar observations were made by Schloessmann[33] Sahli appears to have encountered no small difficulty in explaining the persistent bleeding in spite of the normal coagulation time of the blood. Several observers have denied that this is a regular finding. A comment on these observations is to follow.

The observations of Case 8 are characteristic for the type usually pictured when hemophilia is described. The patient was the youngest of the entire group and also had the most severe hemorrhage. It is a prevalent opinion that hemophilia tends to become milder as the patient grows older. The present group is entirely too small to prove or disprove this statement. It may be pointed out, however, that the two men past 40 years of age (Cases 1 and 6), in spite of their hemophilia, were not prevented from leading an active life, one as a farmer, the other as an executive. In spite of the relatively mild course of the disease during later years, the changes in the coagulability of their blood were pronounced, although not extreme.

Attention will finally be directed to the effect of the blood transfusion in Case 4 The transfusion was considered indicated because of the marked hypocoagulability in this case prior to tooth extraction. The next day the coagulation time of recalcified plasma was within upper limits of normal; after four days it was definitely on the upgrade and still constituted a marked improvement in comparison to the reading before transfusion Eight days after transfusion the coagulation time of the plasma had reached the same value as before transfusion It is of interest to note that the beneficial effect of the blood transfusion in this case of hemophilia appears to be somewhat more protracted as compared to similar investigations in one of our cases of secondary thrombocytopenic purpura (Table XXV).

In Fig. 57 is pictured the coagelgram obtained in Case 4 on Jan. 12, 1940. Of interest, too, is the recorded retraction of the blood clot, a phenomenon normally observed in hemophilia as well as in hemorrhagic cases of jaundice. The coagelgram is included to illustrate the following points. There is nothing about this coagelgram of diagnostic significance, except of course the clot retraction, which would rule out thrombocytopenic purpura. To the observer this coagelgram indicates merely that the velocity of coagulation is reduced as compared to the normal. It does not indicate why the velocity is reduced, whether due to disease or to variations in the investigative technique It does illustrate *how* the coagulability is abnormal in relation to the normal. It is abnormal in two respects; namely, in a relative prolongation of the first as well as the second stage of the process This gives the coagelgram its flattened appearance. As previously stated the velocity of the second stage of the process, the formation of fibrin, is determined by that of the first stage. This is so constant as to constitute a law, an exception to which I have not yet observed. This point is stressed in this connection for one reason As related in the introduction to

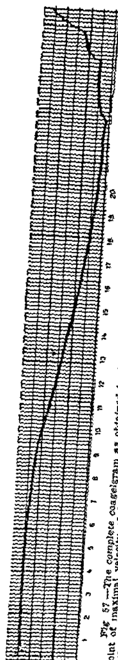


Fig 57 —The complete *coagulogram* as *obtained* in Case 4 (Jan 12, 1940) The lower figures indicate minutes. The point of maximal velocity of fibrin formation (coagulation *time*) is *found* at *about* 13 minutes. Note normal clot retraction at about 23 minutes after onset of the reaction

this chapter, mention was made of the observations of several workers of the prolonged conversion of prothrombin to thrombin in hemophilia, a finding which indicated to them that a qualitative deficiency of prothrombin existed in hemophilia. The reproduced coagelgram indicates their correct observation. The prolonged first stage of the process represents in this case the slow conversion of prothrombin, but so it does in any other case or condition in which the velocity of the entire process is reduced in comparison to the normal. Warner and his colleagues[34] have shown that the rate of prothrombin conversion varies from species to species Brinkhous[29] has recently determined it to be normal in hemophilia The findings of the workers mentioned above, of a relatively slow conversion of prothrombin in hemophilia, constitute just another way of stating that the coagulation time of blood in hemophilia is markedly prolonged.

Experimental Investigation

As the problem now stands, only one type of experiment will be mentioned at this point.

In Fig. 58 the upper curve represents a photo-electric tracing of the process after addition to citrated plasma of an excess of thromboplastin emulsion and a solution of calcium. In reality it is a recording of the prothrombin time according to the regular technique of Quick and indicates that the prothrombin in hemophilia is present in normal amounts. The finding of a normal coagulation time after addition of thromboplastin, or, as Sahli and Morawitz would say, thrombokinase, is nothing but a repetition of the findings of the latter two investigators, who on this basis formed their theory of the defective coagulation in hemophilia as resulting from a quantitative deficiency of thrombokinase.

The two upper tracings of Fig 58 suggested to me a very simple experiment. The prevalent question was this. Can the tracing of the normal prothrombin time in hemophilia be brought to approximate the flattened coagelgram obtained by plain recalcification of the same plasma by simple, gradual reduction of the thromboplastin emulsion? An investigation of this question was carried out twice in Case 4 and once in Case 5.

The experiments gave identical results. One such series of determinations is presented in Table XXII A significant part of the investigation is the selec-

TABLE XXII

RESULTS OF THE VARYING VELOCITY OF REACTION IN A HEMOPHILIAC AND IN A NORMAL PERSON AFTER RECALCIFICATION OF PLASMA TO WHICH WAS ADDED DECREASING CONCENTRATIONS OF AN EMULSION OF THROMBOPLASTIN

SUBJECT	PROTHROMBIN CONTENT IN UNITS PER C.C. OF PLASMA	COAGULATION TIME OF RECALCIFIED PLASMA (SECONDS)	COAGULATION TIME (SECONDS) — CONCENTRATION OF THROMBOPLASTIN (%)								
			100	5	1	0.5	0.1	0.05	0.01	0.001	(H_2O ONLY)
Normal	322	180	10	12	19	27	35	40	50	70	110
Hemophiliac	335	1,520	11	14	28	33	48	70	95	240	460

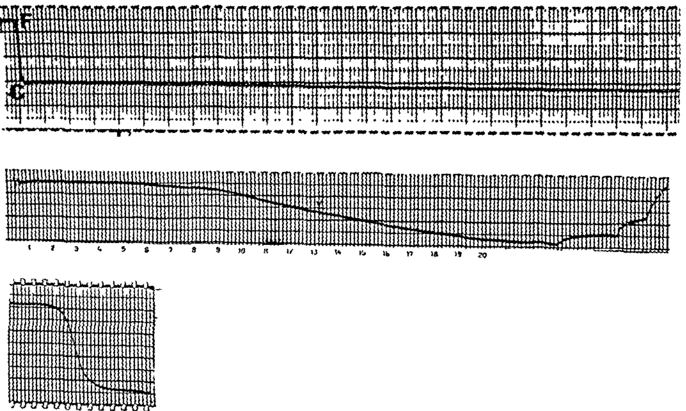


Fig 58 —Reproductions of three photo-electric tracings, which, viewed together, give a good demonstration of the pathologic blood coagulation in hemophilia (middle tracing same as preceding figure). Lower tracing that of recalcified plasma from normal individual. Upper tracing indicates normal prothrombin time (as in hemophilia).

tion of a normal control of a prothrombin level not exceeding that of the hemophiliac's level. The content of prothrombin in both the control and the hemophilic specimen was *determined according to the two-stage technique of Warner* and associates with the modifications necessitated by the photo-electric technique.

It will be noted that there is only a slight difference between the velocity of the reaction of the normal and the hemophilic specimens with thromboplastin of concentrations between 100 and 0.5 per cent. An increasing difference is apparent below the latter concentration Even at concentrations of thromboplastin as low as 0 001 per cent, however, there is a definite influence of thromboplastin still present in both specimens, indicating the high potency of the original 100 per cent emulsion of thromboplastin. In passing, it is worth mentioning that such a dilution experiment may possibly be employed as a means by which to standardize the relative potency of the various tissue extracts which with increasing frequency are employed in the study of various phases related to the problem of coagulation of the blood.

In interpreting the findings in Table XXII, it is necessary to keep in mind that an almost identical difference can be produced by two specimens of different prothrombin level This possibility is ruled out by the selection of normal controls of a prothrombin level not above that of the hemophiliac's level.

Briefly, the experiments have yielded this information· With decreasing concentrations of an emulsion of thromboplastin the velocity of the reaction is relatively more reduced in the cases of hemophilia as compared to normal cases. How is this difference brought about?

Referring again to our previous investigations of the factors governing the rate of the reaction, any one of three factors must be considered; namely, the quantity of prothrombin, of calcium, or of thromboplastin By the execution of the experiment the prothrombin factor is excluded. It is further difficult to understand that the present experimental setup can have rendered the calcium less active in the hemophilic plasmas as compared to normal plasmas. As to the third possibility, we admittedly have added the thromboplastin in identical quantities to normal plasma as well as to the plasma of hemophiliacs This quantity, however, is not the only thromboplastin available in the reacting system Thromboplastin is also furnished by the genuine plasma itself. The latter is to be added to the externally introduced part in order to give the total *quantity of thromboplastin active during the process* On the basis of the relatively more pronounced reduction in velocity of the reaction in our hemophiliac cases with reducing concentrations of thromboplastin, it appears that the total quantity of thromboplastin in the hemophiliac's plasma is less than that of the normal. By simple mathematics this would indicate that the thromboplastin, as furnished by the hemophiliac plasma, is quantitatively deficient in relation to the normal, and that under the present experimental setup this difference is observable only at concentrations of added thromboplastin low enough not to obscure the existing quantitative difference The observations indicate that *a quantitative deficiency of thromboplastin in the hemophilic plasma produces a*

reduced velocity of coagulation which reasonably explains the defective mechanism of coagulation as characteristic of hemophilia.

In view of the more elaborate technique of recent years in isolating the quantitative deficiency, the approach used here must appear rather primitive. Howell's[2] report concluding with his above-mentioned concept was not available to me at the time the experiments were performed. It is of interest here to mention that Addis[5] employed an investigative technique practically identical to the one described above. The observations recorded here are in general agreement with his observations, which, however, due to the potency of his tissue extracts, record differences in minutes between hemophilic and normal plasma where I have recorded only seconds. This is of no consequence in a controlled experiment. The interesting part of his report is his conclusion, which refuted the theories of Sahli and Morawitz. Because of the coagulant effect of tissue extracts in hemophilic plasma, even at very reduced concentrations of the extracts, Addis concluded that this indicates the impossibility of thrombokinase as having anything to do with the defective mechanism in hemophilia. Detailed studies of the effect of various concentrations of thromboplastin emulsion in normal and abnormal conditions naturally facilitate the present detailed analysis and evaluation of such an observation.

Particular mention will be made of the observation as listed in Table XXII that the addition to the control of distilled water or saline solution, in volumetric quantities equal to that of the thromboplastin emulsion, upon addition to citrated plasma increases the velocity of the reaction This is true for the normal as well as for the hemophilic plasma, although the coagulant effect of pure water or saline solution is more pronounced in the latter. This finding is unexpected It is not due to a dilution of calcium. The concentration of calcium chosen for recalcification of the plasma is the one producing the maximal coagulant effect The mere dilution of either of the active factors in reality would be expected to slow down instead of, as noted, to increase the velocity of the reaction. At present I am not prepared to explain this observation satisfactorily. I am inclined, however, to look upon it in the light of the observations of Opitz and Zweig,[27] from whose experiments it appears that the platelets under certain conditions may be observed to liberate their content of thromboplastin (in hemophiliacs, for instance, by merely leaving the plasma standing for several hours). A similar increase in the velocity of the hemophilic recalcified plasma, as I have noted, may also be produced by vigorous shaking of the plasma before recalcification

Comment

In commenting upon the findings reviewed here I *feel obliged* to remind my readers of the introductory sentence to this chapter. Nothing can apparently be taken for granted and surprises may still be presented It is with more than usual interest that further investigation into the problem along the lines followed by Howell will be expected Even if his concept is accepted, there are two points which, without particular consideration, may cause no small and unnecessary confusion These will be touched upon in the following

The investigations of Patek and his colleagues,[17, 18] Bendien and van Creveld[11] and Howell[2] indicate that hemophilia possesses in reduced quantity the active coagulant principle, which Howell interprets as thromboplastin. Many investigators, admitting a qualitative deficiency of platelets, found the thromboplastin in hemophilia to be present in normal quantities. It is thus to be remarked about the latter findings that the thromboplastin was assayed according to its ability to influence the coagulation of blood. It is reasonable to assume that the blood disposes of a certain surplus of thromboplastin, in the same manner as it exhibits a definite surplus of prothrombin. It will then be understood that thromboplastin above a certain minimal quantity may exert a normal coagulant effect without being present in normal quantities.

This is only one side of the picture. The other still hides the secret to the nature of hemophilia The investigations of Fonio, of Minot and Lee, and of others strongly point to an actual functional derangement of the platelets. The technique of Patek and co-workers, comprising a precipitation of the coagulant principle in *filtered or nonfiltered plasma, naturally permits of no conclusions* as to the presence or absence of such a qualitative deficiency of the platelets. The technique as employed by me in the above-considered investigation is further of no consequence for the present question, except to indicate the extreme readiness with which this functional deficiency, if present, may be corrected in the presence of even minute quantities of thromboplastin. But so do, in a more physiologic manner, the observations of Opitz and Zweig. It actually appears that the functional activity of the platelets may be determined by the quantity of thromboplastin available

Certain findings in the present work seem to indicate that a mere quantitative deficiency of thromboplastin hardly suffices for the understanding of the extreme hypocoagulability of hemophilia Reference may be made to the investigated cases of thrombocytopenic purpura in which the hypocoagulability appears to be brought about by a quantitative deficiency of thromboplastin through the thrombocytopenia. Even in extreme cases of thrombocytopenia, however, one never observes the extreme hypocoagulability of hemophilia. It is not inconceivable that the individual hemophilic platelet might not contain more than, say, one-tenth that of the normal or the thrombocytopenic platelet. On the basis of the actual number of platelets in hemophilia and in a case of thrombocytopenic purpura of perhaps 30,000 platelets per cubic millimeter of blood, it would have to contain considerably less, on the basis of direct comparison of the coagulability of the blood in the two conditions, as stated above. But, if the hemophilic platelet contained considerably less thromboplastin, then it seems well nigh impossible to understand how such a specimen might improve its coagulability in vitro upon standing

It appears to me that there is consequently good reason at present to adopt as a working hypothesis the view that the defective mechanism of coagulation in hemophilia is brought about by a combination of a qualitative deficiency of the platelets and a quantitative deficiency of thromboplastin

reduced velocity of coagulation which reasonably explains the defective mechanism of coagulation as characteristic of hemophilia.

In view of the more elaborate technique of recent years in isolating the quantitative deficiency, the approach used here must appear rather primitive. Howell's[2] report concluding with his above-mentioned concept was not available to me at the time the experiments were performed. It is of interest here to mention that Addis[5] employed an investigative technique practically identical to the one described above. The observations recorded here are in general agreement with his observations, which, however, due to the potency of his tissue extracts, record differences in minutes between hemophilic and normal plasma where I have recorded only seconds. This is of no consequence in a controlled experiment. The interesting part of his report is his conclusion, which refuted the theories of Sahli and Morawitz. Because of the coagulant effect of tissue extracts in hemophilic plasma, even at very reduced concentrations of the extracts, Addis concluded that this indicates the impossibility of thrombokinase as having anything to do with the defective mechanism in hemophilia. Detailed studies of the effect of various concentrations of thromboplastin emulsion in normal and abnormal conditions naturally facilitate the present detailed analysis and evaluation of such an observation

Particular mention will be made of the observation as listed in Table XXII that the addition to the control of distilled water or saline solution, in volumetric quantities equal to that of the thromboplastin emulsion, upon addition to citrated plasma increases the velocity of the reaction This is true for the normal as well as for the hemophilic plasma, although the coagulant effect of pure water or saline solution is more pronounced in the latter This finding is unexpected It is not due to a dilution of calcium. The concentration of calcium chosen for recalcification of the plasma is the one producing the maximal coagulant effect The mere dilution of either of the active factors in reality would be expected to slow down instead of, as noted, to increase the velocity of the reaction At present I am not prepared to explain this observation satisfactorily I am inclined, however, to look upon it in the light of the observations of Opitz and Zweig,[27] from whose experiments it appears that the platelets under certain conditions may be observed to liberate their content of thromboplastin (in hemophiliacs, for instance, by merely leaving the plasma standing for several hours) A similar increase in the velocity of the hemophilic recalcified plasma, as I have noted, may also be produced by vigorous shaking of the plasma before recalcification.

Comment

In commenting upon the findings reviewed here I feel obliged to remind my readers of the introductory sentence to this chapter. Nothing can apparently be taken for granted and surprises may still be presented. It is with more than usual interest that further investigation into the problem along the lines followed by Howell will be expected Even if his concept is accepted, there are two points which, without particular consideration, may cause no small and unnecessary confusion These will be touched upon i[illegible]

It is contended that the defective coagulation in hemophilia is not solely explained on the basis of a quantitative deficiency of thromboplastin but most likely combined with an additional factor as represented by a qualitative deficiency of the platelets.

Suggested Test for Estimating Variations in Quantities of Thromboplastin

Anyone interested in investigation of the mechanics of blood coagulation from a theoretic or a practical viewpoint may have felt the urgent need of a practical test for estimation of difference in quantities of thromboplastin. Such tests are not available at the present time. From Howell's latest report it appears that his technique may form the basis of a quantitative technique.

I believe that the technique as employed in the preceding investigation of the deficient factor in hemophilia may also present possibilities for such a test. The suggestion is outlined in direct connection with the investigative approach considered above as it is directly suggested and readily understood in connection with these investigations. At present I have personally no occasion to follow up this suggestion with actual investigation. The following reasoning may be of consequence:

Let us again consider the coagelgram of plainly recalcified plasma in a hemophiliac (Fig. 58). By the addition of a 100 per cent emulsion of thromboplastin the velocity of the reaction in this specimen is increased to the normal velocity with the standard technique employed. If our hemophilic plasma in addition to its quantitative deficiency of thromboplastin also contained less prothrombin, the addition of the thromboplastin emulsion would have resulted in a coagulation time which would be longer than normal, thus making it impossible to decide by one single reading whether the prothrombin or the thromboplastin was the deficient factor. This possibility of variation in the quantity of prothrombin must and can be avoided by the addition to the citrated plasma of a stronger solution of prothrombin. Under otherwise standard conditions the difference in velocity of the reaction upon recalcification of the plasma with prothrombin in excess would then be a relative expression of the difference in quantities of thromboplastin From the above experiment it is apparent that an addition of potent emulsions of thromboplastin would obscure possible variations in quantities of thromboplastin. For this reason, no extra thromboplastin is to be added to the system. This is more than a postulate, as it may be directly observed from the results as given in Table XXII.

By a simple titration technique as outlined for the one-stage titration of prothrombin in plasma or serum, it would then be possible to proceed with dilution of the plasma until a sample giving the arbitrarily chosen fixed coagulation time was obtained By further computation on the basis of the results of the titration, the thromboplastin may be expressed in units per cubic centimeter of genuine plasma

Aside from its clinical importance in the investigation of hemophiliacs, a test of the nature suggested may be of consequence in the further investigation

With this view in mind let us again consider a few of the observations in our series.

The finding of only moderate hypocoagulability of the blood after prolonged bleeding suggests that *this temporary improvement may have been brought about* through absorption into the blood stream of tissue fluids incidental to the process of restoration of the posthemorrhagic blood volume. As to the coagulant effect of tissue extracts in hemophiliacs, this question might appear settled with the works of Gressot.[23] With the new technique for investigation and determination of tissue thromboplastin as employed by Howell and with later information regarding the quantitative assay of thromboplastin, it may prove worth while once more to repeat the investigations of Gressot. The excess of thromboplastin may have obscured the quantitative differences when tested by its effect in a coagulating system, as demonstrated by Table XXII

It will be noted that in all cases of the present series with a moderate hypocoagulability as measured by the recalcification of plasma, a coagulation time of whole blood within or bordering on the upper limits of normal is noted. There is good reason to believe that in hemophilia, as in cases of thrombocytopenic purpura, extrinsic factors like the glassware used during determination of the coagulation time of whole blood may result in normal readings; whereas, the coagulability of blood plasma definitely indicates the presence of a moderate defect in the mechanism of coagulation.

Summary

In cases of hemophilia the coagulability of blood plasma may vary from the most excessive hypocoagulability to be found in any of the hemorrhagic diathesis groups to a hypocoagulability moderate enough to cause a normal or practically normal coagulation time of the whole blood, as measured by routine methods. The diagnosis of hemophilia, therefore, must be based on additional hematologic as well as on clinical information

In the individual hemophiliac are noted cyclic changes in the coagulability of blood plasma which occur spontaneously and for reasons as yet unknown. Prolonged hemorrhage may occasionally induce a marked improvement in the coagulability This may possibly be brought about through an excessive absorption of tissue juices into the blood stream.

The coagelgrams in cases of hemophilia demonstrate that the reduced velocity of the process of coagulation concerns the first as well as the second stage of the process This is a physiologic rule: reduced velocity of coagulation in experimental as well as clinical conditions results from a reduced velocity of thrombin formation which in its turn determines the velocity of fibrin formation.

The coagelgrams in cases of hemophilia demonstrated the presence of a normal retraction of the clot

Experimental findings have been presented which indicate that the hypocoagulability in cases of hemophilia results from a quantitative deficiency of thromboplastin, thus substantiating Howell's interpretation of the findings of Patek and his colleagues and Bendien and co-workers.

23 Gressot, E.: Zur Lehre von der Hämophilie, Ztschr. f. klin. Med. 76: 194 218, 1912.
24. Lowenburg, Harry, and Rubenstone, A. I.: Hemophilia; Experimental Data Bearing on the Effect of Glycerinized Extracts of Visceral Hemophilic Tissue on the Coagulation Time of Blood, J. A. M. A. 71: 1196 1198, 1918.
25 Fonio, A.: Ueber die Gerinnungsfaktoren des hamophilen Blutes. Eine Studie über die Gerinnungsvorgänge, Mitt. a d. Grenzgeb. d. Med. u. Chir. 28: 313-348, 1914.
26. Fonio, A.: Ueber vergleichende Blutplättchenuntersuchungen. Ein Beitrag zur Frage der Methodik der Gerinnungsbestimmungen, Cor.-Bl. f. schweiz. Aerzte. 45: 1505-1524, 1915.
27. Opitz, Hans, and Zweig, Hedwig: Die Hämophilie kein örtliches Gerinnungsproblem sondern eine universelle konstitutionelle Frage, Jahrb. f. Kinderh. 107: 155-174, 1924.
28. Opitz, Hans· Über Hämophilie, Ergebn. d inn. Med. u. Kinderh. 29. 628 685, 1926.
29. Brinkhous, K. M.· A Study of the Clotting Defect in Hemophilia; the Delayed Formation of Thrombin, Am J. M. Sc. 198: 509-516, 1939.
30. Eagle, Harry: Studies on Blood Coagulation. I. The Rôle of Prothrombin and of Platelets in the Formation of Thrombin, J. Gen Physiol. 18: 531 545, 1935
31. Bendien, W. M , and van Creveld, S : On Some Factors of Blood Coagulation, Especially With Regard to the Problem of Hemophilia, Acta med. Scandinav. 99: 12 27, 1939.
32 Morawitz, P.· Die Chemie der Blutgerinnung, Ergebn. d Physiol. 4: 307-422, 1905.
33. Schloessmann, H.. Die Hamophilie Neue Deutsche Chirurgie, Stuttgart, 1930, Ferdinand Enke, vol. 47, 306 pages.
34. Warner, E D, Brinkhous, K M., and Smith, H. P.: The Prothrombin Conversion Rate in Various Species, Proc. Soc Exper. Biol. & Med 40 197 200, 1939.

of, for instance, cases of thrombosis and embolism. I have noted on several occasions a rather pronounced increase in the coagulability of blood plasma by simple recalcification technique.

It is readily understood that the suggested test would require the use of a prothrombin solution of constant, standardized potency, in the same manner as the present test for prothrombin determinations is entirely dependent upon the availability of an emulsion of thromboplastin of the same characteristics.

References

1. Bulloch, W., and Fildes, P.: Quoted by Howell, W. H.[2]
2. Howell, W. H.: Hemophilia; the Wesley M. Carpenter Lecture, Bull New York Acad Med. 15: 3-26, 1939.
3. Sahli, H.. Ueber das Wesen der Hamophilie, Ztschr. f. klin Med. 56: 264 315, 1905.
4. Sahli, Hermann· Weitere Beitrage zur Lehre von der Hamophilie, Deutsches Arch. f. klin Med. 99: 518-556, 1910.
5. Addis, T.: The Pathogenesis of Hereditary Haemophilia, J. Path. & Bact. 15. 427 452, 1911.
6 Klinger, R.: Studien uber Hamophilie, Ztschr. f. klin. Med 85: 335-374, 1918
7. Wohlisch, Edgar· Untersuchungen zum Hamophilieproblem, insbesondere zur Thrombocytenfrage, Ztschr. f d. ges exper. Med. 36: 3-21, 1923.
8. Hess, A. F.: The Calcium Factor in Hemophilia. A Case With Calcium Deficiency (Calcipriva), Bull. Johns Hopkins Hosp. 26: 372-376, 1915.
9. Howell, W. H: The Condition of the Blood in Hemophilia, Thrombosis and Purpura, Arch. Int Med. 13: 76 95, 1914.
10. Morawitz, P., and Lossen, J · Über Hamophilie, Deutsches Arch. f. klin. Med. 94· 110-124, 1908
11. Nolf, P., and Herry, A.· De l'hémophilie; pathogénie et traitement, Rev. de méd, Paris 30. 19-40, 107-125, 1910.
12. Minot, G. R., and Lee, R. I: The Blood Platelets in Hemophilia, Arch Int. Med. 18: 474-495, 1916
13. Howell, W. H: Note Upon the Presence of Heparin in Normal and Hemophilic Blood of Man, Am. J Physiol 77: 680-687, 1926
14 Howell, W. H., and Cekada, E B The Cause of the Delayed Clotting of Hemophilic Blood, Ibid 78: 500-511, 1926.
15. Christie, R. V., Davies, H W, and Stewart, C P: Studies in Blood Coagulation and Haemophilia. II. Observations on Haemic Functions in Haemophilia, Quart. J. Med. 20: 481-498, 1927
16 Quick, A. J.: The Prothrombin in Hemophilia and in Obstructive Jaundice, J. Biol. Chem. 109: lxxxiii-lxxxiv, 1935.
17. Patek, A. J., and Stetson, R. P · Hemophilia. I. The Abnormal Coagulation of the Blood and Its Relation to the Blood Platelets, J. Clin Investigation 15: 531-542, 1936.
18. Patek, A. J., Jr., and Taylor, F. H L. Hemophilia. II. Some Properties of a Substance Obtained From Normal Human Plasma Effective in Accelerating the Coagulation of Hemophilic Blood, Ibid. 16: 113-124, 1937
19. Dam, H., and Glavind, J.· The Clotting Power of Human and Mammalian Blood in Relation to Vitamin K, Acta med Scandinav 96· 108-128, 1938
20. Feissly, R., and Fried, A Die Blutplattchen des hamophilen Blutes, Klin Wchnschr 3: 831-834, 1924.
21. Frank, E., and Hartmann, E. Über das Wesen und die therapeutische Korrektur der hämophilen Gerinnungsstorung, Ibid 6: 435-439, 1927.
22. Schloessmann, H: Studien zum Wesen und zur Behandlung der Hamophilie, Beitr. z. klin. Chir 79: 477-549, 1912.

itself. Its main clinical characteristics are the hemorrhagic manifestations; that is, the extravascular accumulation of blood. It is a deplorable handicap that the organ actually concerned in this abnormal process, namely, the vascular tree, still is somewhat outside the range of more accurate observation and investigation because of lack of adequate methods The peculiar situation has arisen that our main approach to this disorder has been by hematologic methods. Whether hematologic information has been positive or negative, the probable natural tendency has been to make conclusions as to the functional activity of the vascular tree without possessing means by which these assumptions may be verified.

The lack of investigative methods has made it increasingly difficult to present clinical and experimental studies concerning this disorder without repetition of only too well-known observations which add little to the clarification of the problem. During the subsequent exposition I know I personally shall be too aware of these limitations as set by the investigative technique. I believe that these limitations hide from our view an important field which necessarily has to be directly attacked by future research in order to be able to progress further. To justify this belief, the subsequent exposition requires treatment of information yielded by the available clinical material in a manner admittedly not strictly academic. At present, however, it appears to me of some consequence to depart from the secure ground of clear-cut evidence to the dangerous sphere of hypothesis in an attempt to scan the field for future approaches to hard-needed facts

Material

The clinical material investigated consisted of twenty-eight patients, the condition of whom satisfied the general requirements for a definite diagnosis of essential thrombocytopenic purpura. A preliminary report has been presented of findings in seventeen of these cases.[21] The eleven patients comprising the remainder of the group were subjected to splenectomy The investigations also included eleven cases with thrombocytopenia in conditions other than essential thrombocytopenic purpura.

The bleeding time was determined according to the method of Duke Where determinations of the coagulation time of whole blood were undertaken, the method of Lee and White[23] was employed The counting of the number of platelets was performed according to a technique previously described[25] In practically all instances the enumeration of platelets was undertaken either by the chief technician of the laboratory or myself

Results

1 **The Coagulability of the Blood Plasma in Essential Thrombocytopenic Purpura**—In Table XXIII an account is given of some hemorrhagic investigations of particular interest in seventeen cases of essential thrombocytopenic purpura It will be noted that the hematologic features commonly recognized as characteristic of this disorder were found in all. All of the cases had a

CHAPTER XI

THROMBOCYTOPENIC PURPURA

In an excellent review by Jones and Tocantins[1] of the history of purpura the reader is left with an impressive picture of centuries of effort behind the confusing and multitudinous hemorrhagic manifestations, to see the play of clinical entities through various etiologic principles and pathogenetic processes. Today, reviewing the wealth of accumulated data, we are still confused by these many conditions. We see clearly enough the justification of separating the symptom of hemorrhagic manifestations into various clinical entities. We have accepted the clinical entity as described by Werlhof[2] and by Behrens[3] in 1735 and known to us as morbus maculosus Werlhofii; or according to the terminology of Wilan, purpura hemorrhagica; or according to Frank, essential thrombocytopenic purpura We agree with the findings of Krauss[4] (1883) and Denys[5] (1887), later verified by Hayem[6] (1890), that some of the essential findings of hemorrhagic purpura are the abnormally low number of platelets, the absence of retraction of the blood clot (Hayem[7] [1896]), the abnormally prolonged bleeding time (Duke[8] [1912]), and the decreased capillary resistance (Frugoni and Giugni,[9] Leede,[10] Weill and Chalier,[11] Hess and Fish[12] [1911]). An increasing number of successfully performed splenectomies for hemorrhagic purpura after the suggestion of Kaznelson[13] (1916) has substantiated the justification of surgical intervention in many cases As to the etiology of this disease, however, little is known definitely. As to the pathogenesis, we are still in the midst of the discussion raised by Kaznelson[13] and by Frank,[14-17] the former postulating that the thrombocytopenia is the result of abnormal peripheral destruction through the activity of the spleen, while Frank maintained that the thrombocytopenia resulted from a compromised thrombocytopoietic activity of the bone marrow At present it can be said that the question has not been settled in favor of either viewpoint.

Although information is not lacking to the contrary, it seems now that the megakaryocytic origin of the platelets according to the theory of Wright[18] (1906) is accepted by most hematologists;[19, 20] further that platelets are formed wherever megakaryocytes are present; that is, in the bone marrow, in the spleen and the liver, and, as has been shown by Howell and his associates[21] (1937), in the lungs also.

These are the high points of the development of the present disorder and simultaneously an indication of some of the main criteria for the clinical diagnosis of morbus maculosus Werlhofii. A more complete review of this topic has been given recently by Hemild[22]

For a fuller understanding of this disease it appears that many essential points are still lacking. A reason for this may be sought in the condition

TABLE XXIV

RESULTS OF HEMATOLOGIC EXAMINATION IN 11 CASES OF THROMBOCYTOPENIA

CASE	AGE, YEARS, AND SEX	CLINICAL DIAGNOSIS	NO. OF PLATELETS PER CU MM. OF BLOOD	COAGULATION TIME OF RECALCIFIED PLASMA (SECONDS)	HEMOGLOBIN GM. PER 100 C.C. OF BLOOD	NO OF ERYTHROCYTES PER CU.MM OF BLOOD	NO. OF LEUCOCYTES PER CU.MM. OF BLOOD
18	5 M	Acute bacteremia (diplococcic pneumonia, gr. IV); blood dyscrasia—leucemia?	44,000 41,000	420 530	2.2	960,000	1,400
19	4½ M	Subacute lymphatic leucemia	39,000	360	6 4	2,900,000	13,900
20	34 M	Chronic myelogenous leucemia	60,000	140	13.3	4,400,000	22,700
21	31 M	Aplastic anemia	23,000 —	360 330	6.8	2,400,000	1,600
22	50 M	Bone marrow dyscrasia with involvement of bone marrow	11,300	510	8.5	2,030,000	1,500
23	32 M	Aplastic anemia? (leucemia of peculiar type not excluded)	See Table XXV				
24	62 M	Aplastic anemia?	32,000	310	35 to 65%	1,530,000 to 3,510,000	1,500 to 4,500
25	39 F	Gaucher's disease	49,000	390	10.5	3,800,000	5,000
26	27 M	Anemia megalo splenica	56,000	300	11.9	3,700,000	3,300
27	30 M	Anemia megalosplenica	44,000	420	64%	2,300,000	2,900
28	39 F	Anemia megalo splenica	49,000	420	45 to 66%	3,800,000	11,000

a primary condition other than hemorrhagic purpura. The results indicate that *in most instances of thrombocytopenia, whether of primary or secondary origin, there is a moderate to a more pronounced hypocoagulability of the blood plasma.* This substantiates the finding of Gram,[29] published twenty years ago, a report which appears to have been largely overlooked by subsequent investigators.

Knowing the coagulability of whole blood to be determined by that of the plasma it is obvious that the above conclusion is in contrast to the generally accepted statement[1, 6, 24] *that the coagulability of the blood in this condition is* essentially within normal limits Before this discrepancy is subjected to closer consideration it seems convenient first to search for the explanation of the reduced coagulability of the plasma stated here.

The fact that reduced coagulability of the plasma is observed in such different clinical entities as splenic anemia, essential thrombocytopenic purpura, aplastic anemia, and Gaucher's disease makes it plausible that the explanation is to be found in the factor common for these disorders; namely, the platelets. Several findings support this possibility

A *In the experimental part of this work an account is given of the effect* of centrifugation on the coagulation time of recalcified plasma The coagula-

history of a moderate to a pronounced hemorrhagic tendency. Most of them at the time of examination revealed hemorrhagic manifestations. In all, the tourniquet test indicated lowered capillary resistance. The investigation of the coagulability of the blood plasma in the seventeen cases was undertaken with the visual reading technique. It is to be noted that the average coagulation time of the plasma in normal subjects according to this technique is 195 seconds. In the other cases in the present material the photo-electric reading technique was employed.

TABLE XXIII

RESULTS OF HEMATOLOGIC INVESTIGATION IN 17 CASES OF ESSENTIAL THROMBOCYTOPENIC PURPURA*

CASE	AGE, YEARS, AND SEX		NO. OF PLATELETS PER CU MM	COAGULATION TIME OF RECALCIFIED PLASMA (SECONDS)	COAGULATION TIME OF WHOLE BLOOD (LEE AND WHITE) (MINUTES)	BLEEDING TIME (DUKE) (MINUTES)
1	37	F	6,900	275	9	11
2	52	M	7,425	365	8	9 to 53
			22,800	460	5	40
			8,500	370	4	34
3	13	F	12,500	275	6	5
4	57	M	13,300	245	8	11
5	32	M	15,000	310	8	9
6	34	M	15,000	460	12	60
			23,500	450	—	—
7	44	F	15,400	325	3	30
8	17	F	16,000	370	4	30
9	67	F	16,900	315	9	10
10	52	F	19,000	375	8	30
11	37	M	26,750	380	9 to 11	3½
12	6	F	39,000	340	5	4 to 16½
13	40	F	28,000 to 54,000	265	8	7 to 12½
14	52	F	40,000	335	6	34
15	6	M	46,000	240	6	90
16	53	F	49,000	515	8	16
17	25	F	57,000	320	5	6 to 12½
Normal			320,000	195†	5 to 10	1 to 3

*The clot retraction was absent or markedly delayed in all these cases

†These observations were all based on the test of recalcification of plasma, using the visual reading technique only

From the observations of Table XXIII and the preoperative observations in Table XXVI it will be noted that the coagulation time of recalcified plasma in most of the cases is definitely prolonged. This finding seems so constant as to justify the conclusion that *a moderate to a more pronounced hypocoagulability of the blood plasma is found in most instances in essential thrombocytopenic purpura.*

By employing recalcification of oxalated or citrated *whole blood* the same prolongation of the coagulation time upon recalcification as compared to the normal value is noted. This information is added to indicate that the erythrocytes and the leucocytes are of no direct consequence in this connection.

In Table XXIV are listed the results of various hematologic investigations in eleven cases in which the thrombocytopenia must be considered secondary to

It may appear to be of some consequence in this connection to question whether or not the extravascular sources of thromboplastin may also be depleted in cases of thrombocytopenia. So far as is known no particular investigations on this point are at hand. Indirect evidence suggests that no such depletion exists in these cases. It is a common surgical experience that patients even with almost complete depletion of platelets when operated upon do exhibit no more than normal bleeding during the making of the incision and the instrumental handling of the tissues, in sharp contrast to the profuse hemorrhage from the incised tissues in cases of hemophilia. An abundance of thromboplastic material liberated through the traumatization of tissues as occurs during an incision would explain the experience in the case of thrombocytopenic purpura.

With this information we are now in a position to take up the question: Why is the coagulation time of whole blood, as recorded in our cases, within normal limits as long as the coagulability of the blood plasma is reduced? For an explanation it is necessary to refer to our previous investigations concerning the velocity of the process of coagulation It was determined that the velocity of reaction was governed by three factors, the thromboplastin, the calcium, and the prothrombin The calcium factor can be excluded. Prothrombin is found to be present in normal quantities in this disorder. We are, as shown above, faced with a deficient thromboplastin factor Before we proceed with the argument, the importance of the coagulation time of whole blood when either one of these two latter factors is deficient may be instructively demonstrated

As is to be shown in a subsequent chapter certain cases of obstructive jaundice reveal a more or less pronounced deficiency of prothrombin Several reports in the literature have emphasized the prolongation of the coagulation time of whole blood in jaundiced persons Significantly, before adequate methods were available for determinations of prothrombin, the determination of the coagulation time of whole blood was employed in the detection of the deficient process of coagulation in chicks in which a dietary hemorrhagic condition resulted from a prothrombin deficiency In contrast, the reports on the coagulation time of whole blood in hemorrhagic purpura have almost unanimously stated only normal findings This signifies that a deficiency in prothrombin is of comparatively greater consequence for the velocity of the coagulation as compared to the thromboplastin factor That this is so appears further from the figures of the coagulation time of recalcified plasma in the present cases as compared to those of cases with hemorrhages in obstructive jaundice. The former actually never reaches the height demonstrated in the latter cases A reservation to this statement is brought out in connection with discussion of comparable investigations of patients with hemophilia The comparison between the defect in coagulation of thrombocytopenic purpura and icterus may more correctly be expressed thus the total quantity of prothrombin in jaundiced subjects is reduced to an extent far exceeding the comparable reduction in the thromboplastin factor as regularly found in cases of hemorrhagic purpura, hence the quantitative difference in the defect of the process between the two types of cases. This is also to be expected when it is considered that the platelets represent not the

tion time increases with decreasing number of platelets. Further experiments, previously not dealt with, revealed that the normal coagulability of the plasma could be restored by the addition of platelets from the identical plasma by employing the technique of van Allen[30] for determination of the platelet volume Due to the remarkable suspension stability of platelets in oxalated or citrated plasma the platelets thus added can be considered essentially as representing a suspension and not a solution of platelets. This is a point of some consequence to be considered shortly. In hemophiliacs, Tocantins[31] found that the coagulation time of the blood was further increased after the production of thrombocytopenia through the injection of antiplatelet serum.

B. Several investigators, in spite of reports to the contrary, have presented evidence that platelets do not contain prothrombin (Bordet,[32] Mills,[33] Eagle[34]). It has further been shown[35] that prothrombin is present in normal quantities in essential thrombocytopenic purpura, a fact which we have had occasion to confirm on several occasions. The investigations of Howell[36] revealed that the coagulant factor extracted from tissues by alcohol, the previously mentioned cephalin compound, was able to reproduce and to substitute for the coagulant effect as produced by the platelets. Significant investigations by Chargaff and associates[37] indicated that platelets contain a very potent activator of clotting This factor was found to be contained in the phosphatide fraction which upon further analysis yielded a highly potent cephalin and a less potent lecithin fraction. Cephalins of vegetable origin likewise exhibited high coagulant activity. How far the detailed chemical analysis of this coagulant factor and its purification can proceed is a question of potentialities of analytic methods. At present *the coagulant factor of platelets can be said in many ways to resemble, although not quite identical with that of the tissue-thromboplastin.* From a previous chapter we are familiar with the coagulant effect of tissue extracts as representing the thromboplastic factor in the process of coagulation. The findings presented here strongly indicate that the thromboplastic factor as admittedly present in genuine blood plasma is represented to a definite extent by the blood platelets. In order to avoid misunderstanding it will be stressed that the platelets represent not the only source of the thromboplastic factor It is allegedly found in the endothelial lining of the vessels and in the various extravascular tissues It is readily understood that the extravascular origin of the thromboplastin through the constant interchange between the intravascular and extravascular humors will definitely influence the total quantity of thromboplastin of the plasma This may partly explain the fact that there appears to be no entirely mathematical linear relation between the number of platelets and the coagulability of the plasma This was also noted by Gram,[29] who explained it with the assumption that the platelets may possess varying quantities of coagulant substance. When all the factors mentioned are taken into consideration the impression remains that *the coagulability of the blood plasma in cases with thrombocytopenia is reduced as a result of a reduction of the thromboplastin factor.*

phenomena. At the time of examination at the Clinic he was found to be well developed and well nourished but looked very pale. Scattered areas of ecchymoses were found over the upper and lower extremities as well as in the gums and the buccal and pharyngeal mucosa. Physical examination otherwise gave essentially negative results. It was noted particularly that enlargement of the spleen could not be found. The values for the hemoglobin, erythrocytes, and platelets during the subsequent weeks are listed in Table XXV together with the varying coagulation time of the plasma. The number of leucocytes remained low throughout, ranging between 1,200 and 3,000 with one high reading of 4,000 leucocytes per cubic millimeter. At no time did the blood picture reveal any immaturity in the leucocytes. Occasionally a single normoblast was observed There was a constant relative lymphocytosis, ranging from 53 to 84 per cent with the neutrophiles varying between 19 and 44 per cent. Throughout March, 1935, the relative number of reticulocytes ranged between 1 and 2 per cent; in the following month they increased to about 3 per cent and in May they reached values as high as 12 per cent. The fragility of the erythrocytes on several occasions was found to be within normal limits as well as the serum bilirubin, indicating no increased hemolysis of the erythrocytes.

TABLE XXV

HEMATOLOGIC OBSERVATIONS IN A CASE OF APLASTIC ANEMIA (?) (LEUCEMIA OF PECULIAR NATURE NOT EXCLUDED) (CASE 23, TABLE XXIV)

DATE	BLOOD TRANSFUSIONS (C C)	NO. OF PLATELETS PER CU MM. OF BLOOD	COAGULATION TIME OF RECALCIFIED PLASMA (SECONDS)	HEMOGLOBIN (GM. PER 100 C C. OF BLOOD)	NO. OF ERYTHROCYTES PER CU MM. OF BLOOD	BLEEDING TIME (DUKE) (MINUTES)
1935						
3/4		22,300	—	2.2	1,400,000	40
3/6	500					
3/7		189,000	230	4.4	—	—
3/8		38,000	405	3.46	—	5
3/9	500					
3/11	500					
3/13		50,000	230	5.65	2,230,000	8
3/15	500					
3/20		77,000	420	8.2	2,700,000	—
3/20	500					
3/25	500					
3/30	500					
4/1		35,000	305	9.6	3,140,000	—
4/5	500					
4/20	500					
5/10	500					
5/11		—	270	6.98	2,010,000	
5/17		—	490	—	—	—
5/17	500					
5/20	500					
5/21		42,000	—	8.6	2,090,000	—
5/23	500					
5/25	500	—	—	9.0	3,010,000	—
5/25	Cerebral hemorrhage, death					

The constant and marked anemia in this case, out of all proportion to the insignificant loss of blood through the occasional epistaxis and the subcutaneous ecchymoses, together with the constant leucopenia and the relative lymphocytosis, made a clinical diagnosis of regular essential thrombocytopenic purpura less likely The lack of immaturity of the leucocytes seemed to rule out the possibility of leucemia. Although the impression at first favored the diagnosis of aplastic anemia, the subsequent rise in the relative number of reticulocytes made it increasingly clear that this diagnosis could not be upheld without erratic behavior of the bone marrow response, which is not at all in line with the general experience in this condition. Because of the uncertainty of the diagnosis it was clear that

only source of thromboplastin available to the plasma. It is of interest to note that in less extreme cases of obstructive jaundice a perfectly normal coagulation time of whole blood is found, and this in spite of a definite prothrombin deficiency as ascertained by adequate hematologic methods. This brings out one essential point: *the principle of employing whole blood in the manner regularly used in determination of the coagulation time of the blood is to a given extent defective, as it fails to reveal the presence of a moderate quantitative defect of the process.*

In order to understand this peculiarity it may be convenient to refer to the findings made during the ultramicroscopic observations of the process of coagulation. It was stated that morphologic changes of the platelets occur as an integral part of the progressing process of coagulation. A definite relation was noted between the rapidity with which these morphologic changes took place and the velocity of the entire process of coagulation. It is suggestive that these morphologic changes coincide with the liberation of thromboplastin from the platelets. In oxalated or citrated plasma the platelets are in a state of highly stabilized suspension, thus ruling out any mass destruction of platelets before the addition of calcium. The condition is different when the blood is received, for instance, in a vessel of unprotected glass. Due to the adhesive properties of the platelets a physical factor, as represented by the unprotected glass surface, will cause a rapid destruction of platelets with a ready liberation of the total quantity of available thromboplastin Even if this total quantity is relatively reduced, its rapid release may be sufficient to obscure the more gradual liberation which takes place in the recalcified plasma and thus thwarts the physiologic sequence of events responsible for the velocity of the reaction.

Eagle[34] noted that plasma passed through a Berkefeld filter could be restored to its normal coagulability by the addition of only one-twentieth to one-fiftieth of the original quantity of the platelets Without knowing definitely in which state the platelets were at the time of addition, it is not possible to explain this peculiarity; however, it seems suggestive that the platelets before addition might have undergone preliminary morphologic changes, thus producing an experimental condition not comparable to the condition before the passage through the filter.

That even a precipitated liberation of thromboplastin in many cases is not sufficient to bring about an apparently normal velocity is evident from the fact that, at least in some of the cases of thrombocytopenic purpura, the coagulation time of whole blood actually is prolonged (Evans[38])

In close connection with the previous discussion it may be of interest separately to consider the hematologic observations in Case 23 (Table XXV)

CASE 23—The patient was a farmer, aged 32 years, who registered at the Mayo Clinic March 4, 1935. He had felt perfectly well up to March, 1934, when the onset of spontaneous bleeding from the gums, occasional epistaxis, and the periodic appearance of petechiae and ecchymoses in the skin over various parts of the body had begun. During the summer of 1934 he constantly felt physically weak From then up to his admission to the Clinic the condition gradually and slowly increased without the appearance of other

coagulability changed into one of hypocoagulability with the appearance of the definite thrombocytopenia. From these observations it appears that only a few minutes are required before the liberated coagulant material is inactivated in the peripheral circulation.

Certain observations in the foregoing case are of particular interest in this connection. The blood transfusion given on March 6 raised his number of platelets considerably. On the following day, March 7, it was as high as it ever was recorded (189,000) and simultaneously the coagulability of the plasma was within normal limits. During the next twenty-four hours the count indicates a destruction of approximately 150,000 platelets per cubic millimeter of blood. Significantly the coagulation time of plasma during this period of actual destruction increased from 230 to 405 seconds. I take this to mean that destruction of platelets occurred in great numbers and that the liberated thromboplastin was rapidly removed, thereby terminating the beneficial effect of the blood transfusion, so far as its antihemorrhagic efficacy is concerned, about 48 hours after the transfusion Assuming that this represents the average period of the antihemorrhagic effect of blood transfusion in this case, it will be understood that no accumulative coagulant effect could be obtained by fourteen transfusions over a period of approximately 80 days. In order to have obtained the maintenance of a normal coagulability in this case it would have been necessary to produce platelets to parallel the rate of destruction. With the low number of all the corpuscular elements of the blood in this case it is only reasonable that also the thrombopoietic activity of the bone marrow was involved, making such an increased production impossible This case illustrates that *an important part of the coagulant material of the blood is represented by the total number of the intact, circulating platelets.*

2. The Effect of Splenectomy in Essential Thrombocytopenic Purpura.—Since Schloffer at the suggestion of Kaznelson[13] in 1916 removed the spleen from a patient with essential thrombocytopenic purpura with excellent result, numerous reports in the literature have substantiated the merits of surgical treatment in this condition From the extensive reviews of the experiences of varying surgeons as given by Whipple, Spence, Quénu, W. J Mayo, Giffin, Pemberton, Eliason and Ferguson, and others,[40-53] the impression remains that the rationale of the surgical treatment in this disease is not as clear as was assumed in the early 1920's

In Table XXVI are listed the observations of the number of platelets and the coagulation time of recalcified plasma in eleven cases which fulfilled the requirements for the diagnosis of essential thrombocytopenic purpura. In nine of these cases operation was performed at the Mayo Clinic and in two at the Rikshospitalet Ten of these cases were of the chronic type; on one patient (Case 35) operation was performed during a thrombocytoclastic crisis. No deaths occurred in this group.

According to the surgical response as reflected by the postoperative changes in the number of platelets, the eleven cases may be divided into two separate

splenectomy was not indicated at that time. In the hope that some future development might bring about a clarification of the picture it was decided to follow conservative treatment, consisting essentially of repeated transfusions of blood.

Following the first transfusion the patient felt greatly improved and was able to receive the subsequent transfusions as an ambulant case. Throughout the subsequent course the petechiae, ecchymoses, and epistaxis recurred but somewhat less freely than before.

The patient was seen by the clinician on May 24, 1935, at which time some mild bleeding from the gums was observed. The next morning the patient was brought into the hospital in semistuporous condition, apparently having suffered a cerebral hemorrhage during the night. Marked bleeding from the gums was noted. A large ecchymosis covered his right eyelid. The entire face was sprinkled with petechiae which had not been present on the previous day. He complained of general headache. The right pupil was markedly dilated, while the left was normal. The left arm was flaccid, as was the right arm later in the afternoon. Fresh hemorrhages were noted in the fundi of both eyes. The neurologic diagnosis was intracranial hemorrhage with a possible hemorrhagic encephalitis Attacks of rigidity in the arms with inward rotation suggested a cerebellar convulsion. The patient died in coma the same afternoon

The findings in this case, aside from several points of great hematologic consequence, illustrate some of the pitfalls in the hasty diagnosis of essential thrombocytopenic purpura. There seems little doubt about the final outcome if splenectomy had been performed on this patient. Realizing the extreme diligence with which the clinical diagnosis of essential thrombocytopenic purpura has to be made in a great number of cases, one wonders how many of the fatalities after splenectomy in the so-called acute cases might have been avoided by stricter requirements for a final diagnosis of essential thrombocytopenic purpura. *We believe the requirements for splenectomy were not present in this case. This does not affect the interpretation of the observations concerning the changes in coagulability of the plasma in this case.*

On only three occasions (March 3 and 13 and May 11) did the coagulability of the plasma fall to or within the limits of normal. In the other observations there was a constant and abnormal reduction of the coagulability of the blood plasma, and this in spite of a series of fourteen transfusions This suggests one essential point · *The antihemorrhagic factor as present in blood transfusions does not remain in the circulation for long.* In the present case it could not produce an accumulative effect It appears to have been inactivated or metabolized. This is no doubt a physiologic process It is computed that under normal conditions the platelets remain in the circulation from approximately three to five days before being broken down, further, that the regenerative process occurs at a rate of about 100,000 platelets per cubic millimeter per day. The liberated coagulant material contained in the platelets would have to be inactivated or metabolized, as constantly increasing accumulation of thromboplastic material normally would be incompatible with the maintenance of the fluidity of the blood.

Experimental findings support this view. Tocantins[13] noted after intravenous injection of antiplatelet serum in dogs a period of hypercoagulability during the actual period of platelet destruction, that is, within three to five minutes after the injection of serum Soon, however, this period of hyper-

35	17 F	16 0 (370)				275 (180)								800 (120)				On 19th day: 443 (190)
36	37 M	20 7 (355)	38 (250)		169 (300)			824 (120)		990 (210)					552 (235)		230 (230)	On 18th day: 40 (300) On 23rd day: 27 (255) In 7½ months: 73 (350)
37	52 M	23 75 (480 375)	11 6 (320)			59 (180)					74 (190)				104 (180)		110 (150)	On 22nd day: 43 (170)
38	14 F	20-8 0 (300)	9			54 (150)		69 (140)			44 (---)						58 (160)	On 17th day: 39 (160) On 29th day, 45 (--)
39	37 F	7 0 (330)				28 (163)					51 (---)						15 (300)	In 8 months: 32 (290)

*Only one of these patients was operated upon during the acute stage (Case 35); the operation was performed as an emergency procedure after blood transfusions had failed to stop an excessive hemorrhage from the nose and uterus (menstruation).

TABLE XXVI

OBSERVATIONS OF THE NUMBER OF PLATELETS AND THE COAGULABILITY OF BLOOD PLASMA IN 11 CASES OF ESSENTIAL THROMBOCYTOPENIC PURPURA (OPERATION—SPLENECTOMY)*

CASE	AGE AND SEX	NO. OF PLATELETS (IN THOUSANDS) AND THE COAGULATION TIME (IN SECONDS) OF RECALCIFIED PLASMA (IN PARENTHESES)																
		BEFORE SPLEN-ECTOMY	DAYS AFTER THE SPLENECTOMY															
			WITHIN 2 HR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	LATER
29	14 F	11 32 (?)	31 (270)		118 (120)	130 (165)			353 (170)	(150)			398 (155)					
30	54 M	24 (?)					150 (140)	276 (140)		869 (170)			1,150 (170)			1,016 (185)		
31	54 F	23 (360)			39 (480)	67 (205)	182 (170)	254 --	428 --		772 --				720 (175)			On 19th day: 445 (170)
32	19 F	10 (315)			92 (145)	197 --		244 (150)	224 --			278 (145)					217 (165)	
33	15 F	11 -1 (400 360)	4 5-5 0 (170)				250 (170)		400 (150)			504 (160)					223 (155)	See Table XXVII
34	20 F	17 5 (205)						193 (180)				276 (145)						

TABLE XXVII

OBSERVATIONS OF SEVERAL YEARS' DURATION OF A WOMAN (BORN 1922) WITH ESSENTIAL THROMBOCYTOPENIC PURPURA (CASE 33, TABLE XXVI)

DATE	DAYS AFTER OPERATION	COAGULATION TIME OF RECALCIFIED PLASMA (SECONDS)	NO OF PLATELETS	BLEEDING TIME IN MINUTES	CLINICAL NOTES
1928	--	--	--	--	Spontaneous perforation tonsillary abscess without any excessive hemorrhage
1929 (April)	--	--	--	--	Tooth extraction without much hemorrhage
1930 (Dec.)	--	--	--	--	Onset fever, vomiting, abdominal pain; after one week numerous petechiae noted, severe epistaxis; during following years marked manifestations
1931-1934	--	--	1,000-20,000	15-30	of hemorrhagic purpura (petechiae, ecchymoses, repeated epistaxis)
1934	--	--	11,000	20	
1937	--	--			First menses Jan. 11, 1937, very profuse, lasted 3½ weeks necessitating hospitalization; given transfusions
Jan. 20	--	--	15,000	20	
Jan. 22	--	--	60,000	--	
Feb 6	--	--	1,500	--	
Feb 17	--	--	1,000	40	
June 3	--	--	2,500	20	Following menstruations less severe, of 14 days' duration; menses in June lasted more than a month, very severe, necessitating transfusions
Aug 28	--	--	2,500	--	Profuse menstruation since Sept.; blood transfusions given; family finally consents to operation; preoperative treatment started
Oct. 16	--	--	2,200	--	
Nov. 1	--	400	4,500	20	Specimen taken right before splenectomy
Nov. 1	--	170	5,000	--	Specimen taken ⅙ hour after splenectomy during which blood transfusion was given
Nov. 4	4	170	250,000	4	Petechiae blanching, Lede-Rumpel's sign negative
Nov 6	6	150	400,000	3	
Nov 9	9	160	504,000	3	
Nov 15	15	155	223,000	4	
Nov 18	18	170	86,000	9	
Nov 30	*30*	*170*	*50,500*	*10*	*Lede Rumpel's sign negative; no petechiae* Postoperative course uneventful
Dec 17	48	160	190,000	8	Has had first menstruation after operation, lasted 3 days, scant
1938 Jan. 17	2½ months	145	160,000	5	Is gradually taking up a normal, active life again, no ecchymoses; does not bruise easily even when she is skiing
Feb 21	3¾ months	170	298,000	3	
March 24	4¾ months	160	110,000	5½	
May 5	6 months	*180*	*140,000*	3	Menstruation has been regular and normal; feels very well; never has to take particular precautions
July 29	8 months	165	246,000	4	
1939 Feb 14	15½ months	180	108,000	3	Since operation has had no subjective reminder of previous disease

groups. Cases belonging to Group 1 (Cases 29 to 36 inclusive) exhibited a pronounced postoperative thrombocytosis. In Group 2 (Cases 37, 38, and 39) a small increase was noted in the number of platelets, which did not, however, reach the level of about 320,000 considered the average normal with the technique employed.

The postoperative thrombocytosis characterizing Group 1 is the one most commonly encountered in the most successful cases. A common finding is, further, the secondary decline in the number of platelets following the initial postoperative rise, the decrease occasionally reaching a definite thrombocytopenia of transitory (Case 33) or more permanent nature (Case 36).

The immediate postoperative result in the cases of Group 1 was good The existing preoperative hemorrhagic manifestations gradually disappeared following the operation, the bleeding time became normal, and the capillary fragility returned to normal.

It is evident that the final therapeutic result of splenectomy in these cases can be obtained only by re-examination of the patients at long intervals after the operation. We have succeeded in following two of the cases of Group 1 for a considerable time after the splenectomy.

Our experience in Case 36 may be of interest. From the sixth postoperative day the patient exhibited symptoms of a small pulmonary embolism without manifest thrombosis This is a surprisingly rare complication in these cases when contrasted to the very frequent occurrence of postoperative thrombosis and embolism after splenectomy in cases of megalosplenic anemia. During the third postoperative week a most pronounced reduction in the number of platelets took place, reaching the low level of 27,000 per cubic millimeter of blood on the twenty-third day after operation. At this time no clinical manifestation of a hemorrhagic tendency had reappeared. About seven and one-half months after the splenectomy the platelets numbered 73,000 per cubic millimeter of blood At this time there was a moderate decrease in the capillary resistance as measured by the tourniquet test The patient related that he bruised somewhat easily but in no way as readily as before the operation, and considered himself greatly improved

In Case 33 (Table XXVII) the postoperative thrombocytosis was followed by a transitory thrombocytopenia about the third or the fourth postoperative week. No spontaneous petechiae appeared nor were petechiae produced by the tourniquet test During the following weeks and months the platelets fluctuated between 100,000 and 200,000 per cubic millimeter with constantly normal bleeding time and negative tourniquet tests

The cases of Group 2 are of particular interest as the operation in none of these cases was followed by any remarkable increase in the number of platelets. The platelets in this group may be said to have increased according to a curve resembling that of Group 1 but located on a considerably lower level

Did the postoperative clinical condition in these cases differ from those in which a satisfactory postoperative thrombocytosis took place? In none of these three cases were there any signs of fresh hemorrhagic manifestations

mal, the rate of improvement during the first days roughly paralleling the increase in the number of platelets. After having reached a certain level, however, there appears to be no definite relation during this postoperative stage between the number of platelets and the coagulability of the plasma.

In Group 2 the surprising finding was made that, in spite of a very moderate increase in the number of platelets, the coagulability of the plasma returned to normal in the same way as in Group 1. In two of them (Cases 37 and 38) it remained normal up to 22 and 17 days respectively after the splenectomy. In Case 39, however, the coagulability became definitely reduced again 14 days after the operation.

In the present group of cases it is concluded that, *whereas the coagulability of the plasma in most of these cases is definitely reduced before the operation, the splenectomy in all of them is followed by an immediate return of the coagulability to within normal limits, irrespective of the actual number of circulating platelets. It appears that in some of the cases at least there is a tendency of the coagulability to exhibit a subsequent secondary reduction which lags considerably in time behind the secondary fall in the number of platelets.*

These observations at first sight may appear somewhat contradictory. In discussing the various possibilities we miss observations at longer intervals after the operation. These would admittedly have yielded a safer basis for discussion.

Such observations are available in only three cases. In two of these there was definite thrombocytopenia and prolongation of the coagulation time of plasma 7½ and 8 months, respectively, after the operation (Cases 36 and 39). In Case 33 repeated observations up to 15½ months after the operation revealed the maintenance of a normal coagulability throughout.

These findings suggest that *in the same way as observed in the cases before the operation, the coagulability of the plasma appears to be significantly influenced by the number of circulating platelets when observed after the postoperative processes had readjusted themselves During the first weeks after splenectomy this relation for some reason appears not to exist.*

As long as the theories concerning the mechanism of the therapeutic effect of splenectomy in these cases are as divergent as they appear today, it is admittedly risky to try an interpretation of these latter observations. A reasonable explanation of these observations, however, seems suggestive and is presented with the hope that it may focus interest on certain consequential points

In order to start on firm grounds let it again be stated that we possess accurate information concerning the influence of the thromboplastin factor on the velocity of the process of coagulation as demonstrated in a previous chapter It has further indirectly been indicated that the coagulant effect of the platelets is determined by its thromboplastin activity Under normal conditions this is apparently to a large extent effected through the circulating, discrete platelets Under experimental conditions (e g, intravenous injection of antiplatelet serum) it may also be exerted through the thromboplastin liberated by the peripheral destruction of circulating platelets It has further been pointed

after the operation. From the clinical examination at the time of their dismissal it appeared that these patients were cured. In these cases we fortunately obtained subsequent information, although only one patient was re-examined (Case 39).

In Case 37 a letter received seven weeks after the operation informed us that the patient was gaining and feeling very well. He had noted no recurrence of the previous hemorrhagic manifestations, particularly no reappearance of petechiae over the extremities.

Letters from another patient (Case 38) eight months and one and one-half years, respectively, after the operation indicated that she was able to go skiing and sustain the unavoidable strain and trauma without any signs of ecchymoses or petechiae. Five weeks after the operation her menstrual periods became normal and regular.

Another patient (Case 39) was re-examined eight months after the operation. She related that about seven weeks after operation she had noted the recurrence of fresh crops of petechiae and ecchymoses over her lower extremities, with slight epistaxis on one occasion. These hemorrhagic manifestations, however, improved gradually. At the time of her re-examination the platelets numbered 32,000 per cubic millimeter of blood. The bleeding time was 5½ minutes, a few petechiae appeared after application of the tourniquet, indicating a definitely abnormal fragility of the capillaries The coagulability of the plasma was definitely reduced. She felt greatly improved after the operation and the main reason for returning for an examination was that her last menstrual period had lasted about two weeks and bleeding had been rather profuse.

Summarizing these experiences it may be stated·

Splenectomy in true essential thrombocytopenic purpura is followed by a more or less pronounced immediate increase in the number of platelets, by an immediate return of the bleeding time and the capillary fragility to normal values. In most instances a secondary fall in the number of platelets to normal and subnormal values takes place, the number of platelets finally adjusting itself at a more or less fixed level. In these cases the therapeutic result of the operation must be considered very good. In a smaller group the number of platelets remains after operation at a definitely subnormal level. In these cases one can definitely not speak of the operation as having resulted in a cure of the disorder. The still existing pathologic process, however, appears partly inactivated, resulting in an improvement of the condition. In other true cases of hemorrhagic purpura as recorded in the literature the ultimate result of the splenectomy must in an occasional case be considered a complete failure.

The postoperative changes in the coagulability of the blood plasma in these cases may now be considered

In eight of the cases a definite prolongation of the coagulation time before splenectomy was observed; in one case it was normal (Case 34) and in two cases no preoperative observations were obtained (Table XXVI).

Considering the cases of Group 1 as a whole, it is apparent that within 48 to 72 hours the coagulability of the plasma in all these cases has returned to nor-

mal, the rate of improvement during the first days roughly paralleling the increase in the number of platelets. After having reached a certain level, however, there appears to be no definite relation during this postoperative stage between the number of platelets and the coagulability of the plasma.

In Group 2 the surprising finding was made that, in spite of a very moderate increase in the number of platelets, the coagulability of the plasma returned to normal in the same way as in Group 1. In two of them (Cases 37 and 38) it remained normal up to 22 and 17 days respectively after the splenectomy. In Case 39, however, the coagulability became definitely reduced again 14 days after the operation.

In the present group of cases it is concluded that, *whereas the coagulability of the plasma in most of these cases is definitely reduced before the operation, the splenectomy in all of them is followed by an immediate return of the coagulability to within normal limits, irrespective of the actual number of circulating platelets It appears that in some of the cases at least there is a tendency of the coagulability to exhibit a subsequent secondary reduction which lags considerably in time behind the secondary fall in the number of platelets.*

These observations at first sight may appear somewhat contradictory. In discussing the various possibilities we miss observations at longer intervals after the operation These would admittedly have yielded a safer basis for discussion

Such observations are available in only three cases In two of these there was definite thrombocytopenia and prolongation of the coagulation time of plasma 7½ and 8 months, respectively, after the operation (Cases 36 and 39). In Case 33 repeated observations up to 15½ months after the operation revealed the maintenance of a normal coagulability throughout

These findings suggest that in *the same way as observed in the cases before the operation, the coagulability of the plasma appears to be significantly influenced by the number of circulating platelets when observed after the postoperative processes had readjusted themselves. During the first weeks after splenectomy this relation for some reason appears not to exist.*

As long as the theories concerning the mechanism of the therapeutic effect of splenectomy in these cases are as divergent as they appear today, it is admittedly risky to try an interpretation of these latter observations A reasonable explanation of these observations, however, seems suggestive and is presented with the hope that it may focus interest on certain consequential points

In order to start on firm grounds let it again be stated that we possess accurate information concerning the influence of the thromboplastin factor on the velocity of the process of coagulation as demonstrated in a previous chapter It has further indirectly been indicated that the coagulant effect of the platelets is determined by its thromboplastin activity Under normal conditions this is apparently to a large extent effected through the circulating, discrete platelets Under experimental conditions (e g, intravenous injection of antiplatelet serum) it may also be exerted through the thromboplastin liberated by the peripheral destruction of circulating platelets It has further been pointed

out that the liberated thromboplastin appears rather unstable in the circulating blood and is soon inactivated.

Does this information clarify the seemingly contradictory observations of Table XXVI? It does under *one assumption; namely, that the normal coagulability, as observed,* may be considered produced through the summation of the thromboplastin represented by the circulating platelets and the peripherally destroyed platelets. This is parallel to the hypercoagulability observed immediately after intravenous injection of antiplatelet serum If this is the case, why is the thromboplastin not inactivated during the postoperative course? Inactivation may take place and still produce no secondary phase of hypocoagulability if a constant liberation of thromboplastin occurs This would naturally mean a constant destruction of platelets, furnishing a constant supply of extra thromboplastin. From Bedson's[54, 55, 56] experiments it is known that such destruction occurs after removal of the spleen, whose function in this respect is taken over by the rest of the reticuloendothelial apparatus In order to explain the observations that the circulating platelets are definitely increasing after the splenectomy, an increased peripheral destruction would necessitate an immediate postoperative increase in the thrombocytopoietic activity of the megakaryocytes From the morphologic study of the platelets after splenectomy by stained smears or by observations in citrated plasma it is obvious that a different type of platelet is appearing in the circulation as compared to the preoperative observations *After splenectomy the platelets appear larger and in citrated plasma exhibit an unusually uneven, ragged appearance.* It is further to be remembered that the appearance of larger platelets is one of the early signs of the onset of remission in cases of pernicious anemia after institution of adequate liver therapy, that is, in cases in which it is known that the increase in the erythrocytes and the platelets results from increased activity of the bone marrow Admitting that an increased production of platelets after splenectomy is most probable, *the typical curve for the primary postoperative rise and the secondary postoperative drop, according to this view, would be considered the resultant of the increased platelet production and the simultaneously occurring platelet destruction.* In essence this is a synthesis of the opposite views as held by Kaznelson and Frank. The ultimate result of the splenectomy accordingly would depend upon the level at which would be fixed the equilibrium between the oppositely acting forces, platelet production and platelet destruction.

With this view in mind let us again consider Groups 1 and 2 of Table XXVI The normal coagulability in all cases, irrespective of the number of circulating platelets, would then seem reasonable enough What is of considerably higher interest is the fact that in these three cases of Group 2, in spite of a very moderate increase in the number of platelets, the patients nevertheless lost their hemorrhagic manifestations and during the first three weeks after operation by all clinical signs appeared to be cured If the destructive forces immediately after operation in these three cases exceeded the productive forces to a relatively higher degree than in Group 1, the available total of thromboplastin would nevertheless be about the same, if the rate of production was considered equal in

both groups. In other words, the main difference between the groups would be a relative excess of liberated thromboplastin in Group 2. Is it possible that this may have something to do with the fact that these three patients, in spite of their relatively low number of platelets, still were free of hemorrhagic manifestations?

As stated in the beginning of this chapter it is deplorable that adequate methods for closer study of the functional activity of the vascular system is not available. Particularly in cases like the three here especially considered, the need of such methods is deeply felt What is known by our present armamentarium is that the tourniquet test did not produce any petechiae during the immediate postoperative course. What took place in the endothelial lining of the vessels in these cases we do not know. It is reasonable to believe that these cells represent much more than a passive mechanical lining of the vessels. It further seems rather difficult to rest with the suggestion forwarded that the relation between these endothelial cells and the platelets is one of merely mechanical nature, the platelets through their adhesive function acting as a cementing substance of the intercellular spaces May it be that the biologic activity of the endothelial cells to a certain extent is governed also by the chemical constituents of the platelets or the available thromboplastin of the circulation? May it be that the apparent inactivation or metabolization of the free thromboplastin in circulation is brought about by an assimilation of this substance in the endothelial cells where it is allegedly found to be present?

At any rate experimental and clinical findings indicate that the endothelial cells appear to dispose of a certain surplus of functional resources permitting the normal activity of an intact lining before this function is compromised. Experimentally produced thrombocytopenia in dogs does not instantaneously cause the development of petechial hemorrhages.[39] In the cases in Group 2 (Table XXVI) decreased capillary resistance did not develop during the first three postoperative weeks however, in two of them it was definitely present after a long-standing thrombocytopenia *It appears that thrombocytopenia must persist for a certain length of time before the biologic activity of the endothelial cells is likely to produce definite functional vascular disorders.*

May it be that this apparent reserve of functional activity can be linked to a metabolized reserve of thromboplastic substances? Knowledge concerning vital metabolic processes of these endothelial cells is still beyond our reach. We only know that dietary insufficiency of vitamin C produces hemorrhagic manifestations which by all signs indicate a functional derangement of these cells A study of the mechanism of scorbutic hemorrhagic manifestations may open the field for understanding of essential requirements for the normal functional activity of the endothelial cells

Summary

In most cases of essential thrombocytopenic purpura a moderate to a more marked hypocoagulability of the blood plasma is found. This statement holds true in most instances of thrombocytopenia whether this is of primary or secondary origin.

The hypocoagulability in cases of thrombocytopenia is the result of a quantitative deficiency of the thromboplastin factor.

An explanation is suggested for the discrepancy between these conclusions and the more generally accepted findings that the coagulation time of whole blood in essential thrombocytopenic purpura is within normal limits in most instances It is held that, when blood is exposed to the surfaces of unprotected glass, the adhesive properties of platelets through the influence of an external physical factor will bring about a rapid liberation of thromboplastin, which in its turn essentially governs the conversion of prothrombin to thrombin. Thus is thwarted, so far as time is concerned, the physiologic sequence of events in the process of coagulation.

Evidence has been presented indicating that thromboplastin liberated in the circulation does not as such remain in the circulation but appears soon to be inactivated.

As a consequence the coagulability of the blood plasma appears governed essentially by the coagulant material of the intact, circulating platelets, with reservations to be mentioned below.

Splenectomy was performed in one acute and ten chronic cases of essential thrombocytopenic purpura. No deaths occurred in this series. *Attention has been called to the extreme care that should be exercised in making the clinical diagnosis and deciding on the indication of splenectomy in cases of essential* thrombocytopenic purpura

In the present series it was noted that *the operation was immediately followed by a disappearance of existing hemorrhagic manifestations and by the return to normal values of the bleeding time and the fragility of the capillaries.*

In all of the cases in which operation was performed, a pronounced to a very moderate increase in the number of platelets was noted during the first 1 to 2 weeks after operation, followed by a secondary fall to normal or subnormal values of transitory or more permanent nature *Even in cases of pronounced thrombocytopenia that persisted after the splenectomy a definite clinical improvement was noted.*

The splenectomy effected in all cases of the present series a change from a definite hypocoagulability of the blood plasma before the operation to a normal coagulability during the immediate postoperative course. This change during this particular period occurred independently of the number of circulating platelets. It is suggested that during this period the coagulability, in addition to the circulating platelets, is also governed by liberated thromboplastin which appears to be present in excess, due to an increased destruction of platelets After the immediate postoperative reactions have worn off, the coagulability of the plasma during the subsequent months appears again to be dependent upon the number of circulating platelets in the same manner as before the splenectomy

It is suggested that *the ultimate hematologic and therapeutic effect of the splenectomy is determined by the final equilibrium between factors governing the thrombocytopoietic activity of the megakaryocytes and the thrombocytolytic activity of the entire reticuloendothelial system.*

Attention is directed to the functional activity of the endothelial lining of the vascular tree as possibly governed by still nondefined biologic processes under the influence of at least one known metabolic factor; namely, vitamin C.

References

1. Jones, H. W., and Tocantins, L. M.: The History of Purpura Hemorrhagica, Ann. M. Hist. 5: 349-359, 1933.
2. Werlhof, P. G.: Quoted by Jones, H. W., and Tocantins, L. M.[1]
3. Behrens, R. A.: Quoted by Jones, H. W., and Tocantins, L. M.[1]
4. Krauss, E.: Quoted by Jones, H. W., and Tocantins, L. M.[1]
5. Denys, J.: Études sur la coagulation du sang dans un cas de purpura avec diminution considérable des plaquettes, Cellule 3: 445-461, 1887.
6. Hayem, G.: Du purpura, Presse méd. 3: 233-235, 1895.
7. Hayem, G.: Du caillot non rétractile: suppression de la formation du sérum sanguin dans quelques états pathologiques, Compt. rend. Acad. d. sc. 123: 894-896, 1896.
8. Duke, W. W.: The Pathogenesis of Purpura Hemorrhagica With Special Reference to the Part Played by Blood Platelets, Arch. Int. Med. 10: 445-469, 1912.
9. Frugoni, C., and Giugni, F.: Le "signe du lacet" dans les maladies a manifestation hemorrhagiques, Semaine méd. 31: 25-28, 1911.
10. Leede, C.: Hautblutungen durch Stauung hervorgerufen als diagnostisches Hilfsmittel beim Scharlach, München. med. Wchnschr. 58: 293-295, 1911.
11. Weill, E., and Chalier, J.: Purpura provoqué par hypertension veineuse, Lyon méd. 116: 936-940, 1911.
12. Hess, A. F., and Fish, Mildred: Infantile Scurvy: the Blood, the Blood Vessels and the Diet, Am. J. Dis. Child. 8: 385-405, 1914.
13. Kaznelson, Paul: Verschwinden der hämorrhagischen Diathese bei einem Falle von "essentieller Thrombopenie" (Frank) nach Milzexstirpation. Splenogene thrombolytische Purpura, Wien. klin. Wchnschr. 29: 1451-1454, 1916.
14. Frank, E.: Die essentielle Thrombopenie, Berl. klin. Wchnschr. 1: 454-458, 1915.
15. Frank, E.: Die essentielle Thrombopenie, Ibid. 1: 491-494, 1915.
16. Frank, E.: Aleukia haemorrhagica, Ibid. 2: 961-968, 1915.
17. Frank, E.: Die splenogene Leuko-Myelotoxikose. II. Die Rückwirkung von Milzbestrahlung und Milzexstirpation auf das weisse Blutbild bei Morbus Banti, Ibid. 1: 573-577, 1917.
18. Wright, J. H.: The Origin and Nature of the Blood Plates, Boston M. & S. J. 154: 643-645, 1906.
19. Rosenthal, N.: Blood Platelets and Megacaryocytes, in Downey, Hal: Handbook of Hematology I, New York, 1938, Paul B. Hoeber, Inc., pp. 419-496.
20. Tocantins, L. M.: The Mammalian Blood Platelet in Health and Disease, Medicine 17: 155-260, 1938.
21. Howell, W. H., and Donahue, D. D.: The Production of Blood Platelets in the Lungs, J. Exper. Med. 65: 177-203, 1937.
22. Heinild, Svend: Observations on Essential Thrombopenia (Morbus Maculosus Werlhofii), Acta med. Scandinav. 98: 385-421, 1939.
23. Lee, R. I., and White, P. D.: A Clinical Study of the Coagulation Time of Blood, Am. J. M. Sc. 145: 495-503, 1913.
24. Roskam, Jacques: Globulins et temps de saignement, Compt. rend. Soc. de biol. 84: 844-847, 1921.
25. Nygaard, K. K.: A Direct Method of Counting Platelets in Oxalated Plasma, Proc. Staff Meet., Mayo Clin. 8: 366-370, 1933.
26. Nygaard, K. K.: Coagulability of Blood Plasma, Ibid. 7: 544-547, 1932.
27. Nygaard, K. K.: Coagulability of Blood Plasma; Remarks on the Technic of Its Determination, Ibid. 9: 151-156, 1934.

The hypocoagulability in cases of thrombocytopenia is the result of a quantitative deficiency of the thromboplastin factor.

An explanation is suggested for the discrepancy between these conclusions and the more generally accepted findings that the coagulation time of whole blood in essential thrombocytopenic purpura is within normal limits in most instances. It is held that, when blood is exposed to the surfaces of unprotected glass, the adhesive properties of platelets through the influence of an external physical factor will bring about a rapid liberation of thromboplastin, which in its turn essentially governs the conversion of prothrombin to thrombin. Thus is thwarted, so far as time is concerned, the physiologic sequence of events in the process of coagulation.

Evidence has been presented indicating that thromboplastin liberated in the circulation does not as such remain in the circulation but appears soon to be inactivated.

As a consequence the coagulability of the blood plasma appears governed essentially by the coagulant material of the intact, circulating platelets, with reservations to be mentioned below

Splenectomy was performed in one acute and ten chronic cases of essential thrombocytopenic purpura. No deaths occurred in this series. Attention has been called to the extreme care that should be exercised in making the clinical diagnosis and deciding on the indication of splenectomy in cases of essential thrombocytopenic purpura

In the present series it was noted that *the operation was immediately followed by a disappearance of existing hemorrhagic manifestations and by the return to normal values of the bleeding time and the fragility of the capillaries.*

In all of the cases in which operation was performed, a pronounced to a very moderate increase in the number of platelets was noted during the first 1 to 2 weeks after operation, followed by a secondary fall to normal or subnormal values of transitory or more permanent nature *Even in cases of pronounced thrombocytopenia that persisted after the splenectomy a definite clinical improvement was noted.*

The splenectomy effected in all cases of the present series a change from a definite hypocoagulability of the blood plasma before the operation to a normal coagulability during the immediate postoperative course. This change during this particular period occurred independently of the number of circulating platelets. It is suggested that during this period the coagulability, in addition to the circulating platelets, is also governed by liberated thromboplastin which appears to be present in excess, due to an increased destruction of platelets After the immediate postoperative reactions have worn off, the coagulability of the plasma during the subsequent months appears again to be dependent upon the number of circulating platelets in the same manner as before the splenectomy.

It is suggested that *the ultimate hematologic and therapeutic effect of the splenectomy is determined by the final equilibrium between factors governing the thrombocytopoietic activity of the megakaryocytes and the thrombocytolytic activity of the entire reticuloendothelial system*

51. Doan, C. A.: Splenectomy, Indications, Contraindications, Results, J. Med. 19: 178-183, 1938.
52. Bond, B. F. M.: Thrombocytopenic Purpura. Post Splenectomy, Proc. Roy. Soc. Med. 24: 571-572, 1931.
53. Askey, J. M., and Toland, C. G.: Splenectomy in the Treatment of Hemorrhagic Purpura, *Arch. Surg.* 26: 103-109, 1933.
54. Bedson, S. P.: Blood-Platelet Anti-Serum, Its Specificity and Rôle in the Experimental Production of Purpura, J. Path. & Bact. 25: 94-104, 1922.
55. Bedson, S. P.: The Effect of Splenectomy on the Production of Experimental Purpura, *Lancet* 2: 1117-1119, 1924.
56. Bedson, S. P.: The Rôle of the Reticulo-Endothelial System in the Regulation of the Number of Platelets in the Circulation, Brit. J. Exper. Path. 7: 317-324, 1926.

28. Nygaard, K. K.: Coagulability of the Blood Plasma and Changes in the Number of Platelets in Thrombocytopenic Purpura, *Ibid.* 9: 492-496, 1934.
29. Gram, H. C.: The Coagulation Time of Citrated Plasma on Recalcination, Bull. Johns Hopkins Hosp. 31: 364-368, 1920.
30. Van Allen, C. M.: *Volumetric Measurements of Blood Platelets*, J. Lab. & Clin. Med 12: 282-285, 1926.
31. Tocantins, L. M.: Effets de l'injection du sérum antiplaquettes à trois sujets hémophiles et à un *sujet normal. Action de divers sérums sur le sang "in vitro,"* Sang 10: 582-591, 1936.
32. Bordet, Jules. The Theories of Blood Coagulation, Bull. Johns Hopkins Hosp. 32: 213-218, 1921.
33. Mills, C. A: Do Blood Platelets, Plasma and Tissues Yield Thrombin, or Tissue Fibrinogen? Am. J. Physiol. 95: 1-6, 1930.
34. Eagle, Harry: *Studies on Blood Coagulation. I. The Rôle of Prothrombin* and of Platelets in the Formation of Thrombin, J. Gen. Physiol. 18: 531-545, 1935.
35 Dam, H., and Glavind, J.: The Clotting Power of Human and Mammalian Blood in Relation to Vitamin K, Acta med. Scandinav. 96: 108-128, 1938.
36 Howell, W. H.. The Nature and Action of the Thromboplastic (Zymoplastic) Substance of the Tissues, Am. J. Physiol. 31: 1-21, 1912.
37. Chargaff, Erwin, Bancroft, F. W., and Stanley-Brown, Margaret: Studies on the Chemistry of Blood Coagulation III. The Chemical Constituents of Blood Platelets and *Their Rôle in Blood Clotting, With Remarks on the Activation of* Clotting by Lipids, J. Biol. Chem. 116: 237-251, 1936.
38. Evans, W. H.: The Blood Changes After Splenectomy in Splenic Anaemia, Purpura Haemorrhagica and Acholuric Jaundice With Special Reference to Platelets and Coagulation, J. Path. & Bact. 31: 815-832, 1928.
39. Tocantins, L. M. Experimental Thrombopenic Purpura; *Cytological and Physical* Changes in the Blood, Ann. Int Med. 9: 838-849, 1936.
40. Brill, N. E, and Rosenthal, N.: Treatment by Splenectomy of Essential Thrombocytopenia (*Purpura Hemorrhagica*), *Arch.* Int. *Med. 32: 939 953, 1923.*
41. Giffin, H. Z., and Holloway, J. K.: A Review of Twenty-Eight Cases of Purpura Hemorrhagica in Which Splenectomy Was Performed, Am. J. M. Sc. *170: 186-204,* 1925.
42. Whipple, A. O.. Splenectomy as a Therapeutic Measure in Thrombocytopenic Purpura *Haemorrhagica, Surg., Gynec & Obst 42: 329-341, 1926.*
43. Giffin, H. Z: Splenectomy, Ibid 45. 577-585, 1927.
44. Spence, A. W.: The Results of Splenectomy for Purpura Haemorrhagica, Brit. J. Surg 15: 466-499, 1928
45. Mayo, W. J.: Surgical Aspects of Diseases of the Lymphoid Organs, With Special *Reference to the Spleen: With a Statistical Report of the Results Following* the Removal of the Diseased Spleen in 530 Cases, Coll. Papers, Mayo Clin & Mayo Foundation 21: 631-645, 1929
46. *Quénu, Jean* Les résultats de la splénectomie dans le purpura hémorragique, Rev de chir., Paris 67: 24-39, 1929.
47. Pemberton, J. deJ: *Results of Splenectomy in Splenic Anaemia, Haemolytic Jaundice* and Haemorrhagic Purpura, Ann Surg 94: 755-765, 1931
48. Eliason, E. I, and Ferguson, L. K.: Splenectomy in Purpura Hemorrhagica, Ann Surg. *96: 801-829, 1932.*
49 Brown, D. N., and Elliott, R. H E The Results of Splenectomy in *Thrombocytopenic* Purpura; a Comparative Study of *Ten Cases in Which Splenectomy Was Performed* and Eleven Cases Treated by Conservative Methods, J A. M A 107: 1781 1788, 1936
50 Curtis, G M, Doan, C A., and Wiseman, B. K. Splenectomy for Hemoclastic Crises, Ann. Surg 104: 892-904, 1936.

Further reports[7-9] from the same laboratory indicated the active principle to be present in the fat-soluble, unsaponifiable, nonsterol fraction.

In 1935 Halbrook[10] in hemorrhagic disease of chicks noted a curative effect of 5 per cent dehydrated alfalfa or an equivalent level of an ether extract of alfalfa. Almquist and Stokstad,[11, 12] continuing the work previously performed in the same laboratory by Holst and Halbrook,[4] concluded that "the chemical, physical and physiologic properties of the antihemorrhagic vitamin as elaborated independently in this work, are in close agreement with those of Dam."

In dietary hemorrhagic disease of chicks Schönheyder[8] found a marked prolongation of coagulation during the hemorrhagic stage, returning to normal upon an adequate diet simultaneously with cessation of the hemorrhages. It was concluded that the lack of vitamin K in the food caused a decrease of the clotting-accelerating components of the blood; further, that these were not thrombokinase nor antiprothrombin. Further investigations[13, 14] revealed this factor to be represented by a reduction of prothrombin. With this finding the first important stage of the problem concerning vitamin K may be considered completed and the ground cleared for further investigations

In the investigations of the vitamin K content of various foodstuffs and the potency of various preparations of vitamin K, only biologic methods are employed at present During the early stages this assay was based on the determination of the minimal dosage required for the prevention of hemorrhage in chicks (preventive method)[4, 8] The subsequent, more reliable methods all employ the curative principle determining the minimal dosage which under certain specified conditions reduces the clotting time of the blood from a certain pathologic value to the normal value.[14, 15-17, 19, 20] Whichever method is used, certain fundamental difficulties present themselves The clotting time or the prothrombin level of the blood of chicks depends upon the reserve of vitamin K at the time the chick is hatched, its age at the time of the reading, and the content of vitamin K in the diet[18] With the two factors standardized, the third factor in question can be determined This is done on the basis of determination of the coagulability of the blood From the first part of the present work it is clear that a refined technique for investigation of the coagulability of the blood, whichever method is chosen, includes a consideration of a number of factors in order to obtain reliable results It is obvious that the size of the animal used for assay introduces a number of other obstacles to a completely satisfactory hematologic technique Nevertheless, it appears that at the present stage of the problem much would be gained by a closer consideration of the hematologic technique than is the case at present The quantitative determinations of vitamin K express their results in units It is to be pointed out that the so-called Ansbacher and the Thayer-Doisy units are about twenty and thirty times larger, respectively, than the Dam unit

The vegetable kingdom represents the essential source of vitamin K[7, 8, 12, 15, 21, 22] It is mainly to be found in the green leaves Leaves of the chestnut tree are particularly rich in vitamin K and, as mentioned, alfalfa as well as cabbage and spinach and the green leaves of cauliflower As compared to the green leaves the vitamin K content in flowers, seeds, and roots is considerably

CHAPTER XII

VITAMIN K

Before proceeding to the next group of hemorrhagic conditions it may be convenient at this point to consider separately the antihemorrhagic vitamin K. Its detection, its extraction, purification, and isolation, its physiopathology and therapeutic results form an interesting chapter of recent medical progress The various faces of this development, fitted as it is by now into a fairly clear, many-faceted, although not completed, picture, may serve as another reminder of the truly transparent border existing between the so-called pure sciences and the more practical science of medicine.

During experimental investigations of the sterol metabolism of chicks, Dam[1, 2] observed (1929, 1930) that in chicks kept on a specific diet after a time a hemorrhagic tendency developed with manifest subcutaneous and intramuscular hemorrhages localized on the breast, legs, and wings. These observations were incidental and, to judge from his reports, do not appear to have given rise to further investigations at the time. In studying the requirements of fat-soluble vitamins in chicks, McFarlane and his associates[3] (1931) noted that their animals, when placed on a diet of ether-extracted fish meal, would bleed from 12 to 24 hours following insertion of the identification band into the wing A marked prolongation of the coagulation of blood was noted. Although the interest of these authors appears to have been centered directly on the dietary origin of the hemorrhages, they appear to have been sidetracked by their finding that caseinogen, ether-extracted or not, prevented the development of the hemorrhages when added to the diet Holst and Halbrook[4] in 1933 described a "scurvy-like disease" occurring in chicks kept on a diet of fish meal, ground yellow corn, yeast, ground oyster, and cod liver oil After some time bleeding developed in about 70 per cent of the chicks from pin feathers on the neck, wings, and thighs, sometimes the hemorrhages were subcutaneous and at other times they were intramuscular or intra-abdominal. Significantly it was found that 5 Gm of cabbage fed to the affected animals resulted in their complete recovery They stated "We believe that contrary to previous reports growing chicks may suffer from scurvy due to an absence of vitamin C in the diet."

It is not obvious whether or not these reports prompted Dam to return to his previous observations At any rate, Dam[5, 6] the following year reported on investigations of dietary hemorrhagic disease in chicks and concluded that this represented a new deficiency disease not affected by vitamins A, C, D, B_1, or B_2. The addition of cereals and seeds prevented the outbreak of hemorrhagic manifestations. He concluded that the prevention diet contained an antihemorrhagic factor having the characteristics of a vitamin. He suggested that this factor be tentatively termed vitamin K (*"Koagulations-Vitamin"*).

their antihemorrhagic potency being 1,000 and 600 Thayer-Doisy units, respectively. The physical and chemical properties of these two purifications indicated a quinone structure Further investigations revealed that synthetic products of this group were all limited to the 1,4 series of quinones with 2-methyl-1,4-naphthoquinone as the most potent antihemorrhagic product of the series. Its potency was found to equal that of vitamin K_1 (about 1,000 Thayer-Doisy units), a finding substantiated by Ansbacher and Fernholz.[44] Further investigations concerning the structural formula of vitamin K_1 by different groups of workers (Doisy and associates,[45] Fieser and co-workers[46-51]) have led to the conclusion that it is represented by the formula 2-methyl-3-phytyl-1,4-naphthoquinone. By Fieser and his associates it is estimated that 2-4 μ of this quinone equivalent correspond in its antihemorrhagic activity to 75 mg. of alfalfa.

Regarding the action of vitamin K in the animal organism,[54, 55] there is general agreement that the vitamin is directly concerned with the maintenance of a normal prothrombin level of the blood. It is thus in itself no coagulant substance as addition of vitamin K to blood in vitro is of no consequence.[34] When injected intravenously into animals of low prothrombin level a period of a few hours elapses before the onset of the prothrombin increase, thus making it most likely that the vitamin exerts its antihemorrhagic activity through one or several internal organs which require vitamin K for normal production of prothrombin. A mass of experimental and clinical evidence points to the liver as one organ directly concerned with the maintenance of normal coagulability of the blood These are to be considered in more detail in the next chapter Direct evidence of this relationship between the blood and the liver is presented by Smith and his associates,[56, 57] who found a pronounced reduction of prothrombin of the blood after administration of chloroform and phosphorus to the animal, and by Warner,[58] whose partial hepatectomy procedures in animals resulted in a lowering of prothrombin It is as yet too early to state whether the liver is the only organ producing prothrombin From all evidence it seems justifiable to conclude that this is the organ mainly responsible for the synthesis of prothrombin; further, that this synthesis is dependent upon the maintenance of a certain minimal concentration of vitamin K available for the metabolic processes of the liver No exact information is available as to the quantitative relation between the concentration of vitamin K and the synthesis of prothrombin Although this most stimulating physiologic process is still clouded in mystery, the revelations here related and effected through investigations of vitamin K open new approaches to the study of the physiologic mechanism of prothrombin synthesis.

At present, a series of information is at hand painting a fairly clear picture of the pathologic consequences of lack of vitamin K

The symptoms characteristic for the dietary hemorrhagic disease of the chick can be readily reproduced by an identical dietary regimen in ducklings and young geese; a less marked response is noted in pigeons and canaries.[52] In mammals it is considerably more difficult to produce a similar picture on a

less; tomatoes, for instance, contain about one-tenth that of spinach, while considerably less is found in strawberries, soya beans, peas, corn, carrots, and potatoes. *The synthesis of vitamin K in plants takes place in the light, to a* small or negligible degree in the dark. The animal kingdom represents only *a poor source of vitamin K.* Apparently the reason is that the animal organism does not store vitamin K or, if so, only to a small degree. Liver of the hog, one of the relatively richest *animal sources, contains vitamin K in quantities* equal to that of tomatoes Milk contains little, if any.

A point of particular interest is the finding that vitamin K or a substance of vitamin K-like activity is synthesized through the metabolic processes of certain species of bacteria, among them tubercle bacilli.[23] This finding resulted from early observations that moist fish meal after a while exhibited antihemorrhagic properties as well as the failure to cause hemorrhagic disease in chicks when coprophagy had not been prevented.[18] The bacterial origin of vitamin K further explains certain fundamental differences between various animals when put on a vitamin K-free diet

After Dam, Almquist, and Stokstad had found that the active antihemorrhagic principle was contained in the fat-soluble, unsaponifiable, nonsterol fraction of the extract, further improvements of extraction technique have resulted in increasingly potent extracts.[21-25, 27, 28] A highly purified material obtained by Dam and his colleagues[29] is reported to contain about 20,000 units (Dam) per milligram; that is, about 100,000 times that of dried alfalfa *It is a* clear yellow oil. It contains a combination of carbon, hydrogen, and oxygen but *does not contain nitrogen It is claimed*[29, 30] *to exhibit a characteristic* absorption band with four specified maxima and a specific color reaction; the latter statement, however, has been questioned by subsequent investigators.[31] Because of their finding Dam and associates question the validity of earlier reports[28] concerning the isolation of the vitamin in crystalline form.

A new investigative approach to the problem of the nature of the antihemorrhagic substance was gained through the introduction of synthetic products possessing antihemorrhagic activity. In 1933 Anderson and co-workers[32, 33] isolated a pigment present in the acetone-soluble part of the human tubercle bacillus This substance, termed phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone), was subsequently synthesized[34] Almquist and co-workers[13, 23] had proved the antihemorrhagic effect of certain bacteria, among *them the tubercle* bacillus He and Klose[35] found the same to be true for phthiocol when this *synthetic product* was administered to chicks orally, intramuscularly, or intravenously Highly purified concentrates of alfalfa were shown by them to exhibit a color reaction characteristic of phthiocol[36, 37] Further investigations of a series of synthetic products of the same group indicated the antihemorrhagic potency of phthiocol to lie between the methylnaphthoquinone and the hydroxynaphthoquinone Its potency, however, was considerably below that of the highly purified extracts from alfalfa

A series of investigations from Doisy's laboratory[38-42] indicated *the isolation* of vitamin K_1 from alfalfa, and vitamin K_2 from putrefied sardine meal,

their antihemorrhagic potency being 1,000 and 600 Thayer-Doisy units, respectively. The physical and chemical properties of these two purifications indicated a quinone structure. Further investigations revealed that synthetic products of this group were all limited to the 1,4 series of quinones with 2-methyl-1,4-naphthoquinone as the most potent antihemorrhagic product of the series. Its potency was found to equal that of vitamin K_1 (about 1,000 Thayer-Doisy units), a finding substantiated by Ansbacher and Fernholz.[44] Further investigations concerning the structural formula of vitamin K_1 by different groups of workers (Doisy and associates,[45] Fieser and co-workers[46-51]) have led to the conclusion that it is represented by the formula 2-methyl-3-phytyl-1,4-naphthoquinone. By Fieser and his associates it is estimated that 2-4 μ of this quinone equivalent correspond in its antihemorrhagic activity to 75 mg of alfalfa.

Regarding the action of vitamin K in the animal organism,[34, 55] there is general agreement that the vitamin is directly concerned with the maintenance of a normal prothrombin level of the blood. It is thus in itself no coagulant substance as addition of vitamin K to blood in vitro is of no consequence.[34] When injected intravenously into animals of low prothrombin level a period of a few hours elapses before the onset of the prothrombin increase, thus making it most likely that the vitamin exerts its antihemorrhagic activity through one or several internal organs which require vitamin K for normal production of prothrombin. A mass of experimental and clinical evidence points to the liver as one organ directly concerned with the maintenance of normal coagulability of the blood. These are to be considered in more detail in the next chapter. Direct evidence of this relationship between the blood and the liver is presented by Smith and his associates,[56, 57] who found a pronounced reduction of prothrombin of the blood after administration of chloroform and phosphorus to the animal, and by Warner,[58] whose partial hepatectomy procedures in animals resulted in a lowering of prothrombin It is as yet too early to state whether the liver is the only organ producing prothrombin From all evidence it seems justifiable to conclude that this is the organ mainly responsible for the synthesis of prothrombin, further, that this synthesis is dependent upon the maintenance of a certain minimal concentration of vitamin K available for the metabolic processes of the liver. No exact information is available as to the quantitative relation between the concentration of vitamin K and the synthesis of prothrombin Although this most stimulating physiologic process is still clouded in mystery, the revelations here related and effected through investigations of vitamin K open new approaches to the study of the physiologic mechanism of prothrombin synthesis

At present, a series of information is at hand painting a fairly clear picture of the pathologic consequences of lack of vitamin K

The symptoms characteristic for the dietary hemorrhagic disease of the chick can be readily reproduced by an identical dietary regimen in ducklings and young geese; a less marked response is noted in pigeons and canaries.[32] In mammals it is considerably more difficult to produce a similar picture on a

less; tomatoes, for instance, contain about one-tenth that of spinach, while considerably less is found in strawberries, soya beans, peas, corn, carrots, and potatoes. The synthesis of vitamin K in plants takes place in the light, to a small or negligible degree in the dark. The animal kingdom represents only a poor source of vitamin K. Apparently the reason is that the animal organism does not store vitamin K or, if so, only to a small degree. Liver of the hog, one of the relatively richest animal sources, contains vitamin K in quantities equal to that of tomatoes Milk contains little, if any.

A point of particular interest is the finding that vitamin K or a substance of vitamin K-like activity is synthesized through the metabolic processes of certain species of bacteria, among them tubercle bacilli.[23] This finding resulted from early observations that moist fish meal after a while exhibited antihemorrhagic properties as well as the failure to cause hemorrhagic disease in chicks when coprophagy had not been prevented.[18] The bacterial origin of vitamin K further explains certain fundamental differences between various animals when put on a vitamin K-free diet.

After Dam, Almquist, and Stokstad had found that the active antihemorrhagic principle was contained in the fat-soluble, unsaponifiable, nonsterol fraction of the extract, further improvements of extraction technique have resulted in increasingly potent extracts.[21-25, 27, 29] A highly purified material obtained by Dam and his colleagues[29] is reported to contain about 20,000 units (Dam) per milligram; that is, about 100,000 times that of dried alfalfa. It is a clear yellow oil. It contains a combination of carbon, hydrogen, and oxygen but does not contain nitrogen It is claimed[29, 30] to exhibit a characteristic absorption band with four specified maxima and a specific color reaction; the latter statement, however, has been questioned by subsequent investigators.[31] Because of their finding Dam and associates question the validity of earlier reports[28] concerning the isolation of the vitamin in crystalline form.

A new investigative approach to the problem of the nature of the antihemorrhagic substance was gained through the introduction of synthetic products possessing antihemorrhagic activity In 1933 Anderson and co-workers[32, 33] isolated a pigment present in the acetone-soluble part of the human tubercle bacillus This substance, termed phthiocol (2-methyl-3-hydroxy-1,4-naphthoquinone), was subsequently synthesized[34] Almquist and co-workers[17, 33] had proved the antihemorrhagic effect of certain bacteria, among them the tubercle bacillus. He and Klose[35] found the same to be true for phthiocol when this synthetic product was administered to chicks orally, intramuscularly, or intravenously Highly purified concentrates of alfalfa were shown by them to exhibit a color reaction characteristic of phthiocol[36, 37] Further investigations of a series of synthetic products of the same group indicated the antihemorrhagic potency of phthiocol to lie between the methylnaphthoquinone and the hydroxynaphthoquinone Its potency, however, was considerably below that of the highly purified extracts from alfalfa.

A series of investigations from Doisy's laboratory[38-43] indicated the isolation of vitamin K_1 from alfalfa, and vitamin K_2 from putrefied sardine meal,

their antihemorrhagic potency being 1,000 and 600 Thayer-Doisy units, respectively. The physical and chemical properties of these two purifications indicated a quinone structure. Further investigations revealed that synthetic products of this group were all limited to the 1,4 series of quinones with 2-methyl-1,4-naphthoquinone as the most potent antihemorrhagic product of the series. Its potency was found to equal that of vitamin K_1 (about 1,000 Thayer-Doisy units), a finding substantiated by Ansbacher and Fernholz.[44] Further investigations concerning the structural formula of vitamin K_1 by different groups of workers (Doisy and associates,[45] Fieser and co-workers[46-51]) have led to the conclusion that it is represented by the formula 2-methyl-3-phytyl-1,4-naphthoquinone. By Fieser and his associates it is estimated that 2-4 μ of this quinone equivalent correspond in its antihemorrhagic activity to 75 mg. of alfalfa.

Regarding the action of vitamin K in the animal organism,[54, 55] there is general agreement that the vitamin is directly concerned with the maintenance of a normal prothrombin level of the blood. It is thus in itself no coagulant substance as addition of vitamin K to blood in vitro is of no consequence.[14] When injected intravenously into animals of low prothrombin level a period of a few hours elapses before the onset of the prothrombin increase, thus making it most likely that the vitamin exerts its antihemorrhagic activity through one or several internal organs which require vitamin K for normal production of prothrombin. A mass of experimental and clinical evidence points to the liver as one organ directly concerned with the maintenance of normal coagulability of the blood These are to be considered in more detail in the next chapter. Direct evidence of this relationship between the blood and the liver is presented by Smith and his associates,[56, 57] who found a pronounced reduction of prothrombin of the blood after administration of chloroform and phosphorus to the animal, and by Warner,[58] whose partial hepatectomy procedures in animals resulted in a lowering of prothrombin It is as yet too early to state whether the liver is the only organ producing prothrombin From all evidence it seems justifiable to conclude that this is the organ mainly responsible for the synthesis of prothrombin; further, that this synthesis is dependent upon the maintenance of a certain minimal concentration of vitamin K available for the metabolic processes of the liver. No exact information is available as to the quantitative relation between the concentration of vitamin K and the synthesis of prothrombin. Although this most stimulating physiologic process is still clouded in mystery, the revelations here related and effected through investigations of vitamin K open new approaches to the study of the physiologic mechanism of prothrombin synthesis.

At present, a series of information is at hand painting a fairly clear picture of the pathologic consequences of lack of vitamin K.

The symptoms characteristic for the dietary hemorrhagic disease of the chick can be readily reproduced by an identical dietary regimen in ducklings and young geese; a less marked response is noted in pigeons and canaries[52] In mammals it is considerably more difficult to produce a similar picture on a

dietetic basis, although it can be done in mice[60] *and rats,*[59, 61] and to a certain extent in rabbits[54] It is not definitely shown that lack of vitamin K in the diet can produce hemorrhagic symptoms in adult human beings. The problem of K-avitaminosis in newborn infants is to be taken up in a subsequent chapter. The difference noted between fowls and mammals in this respect is striking. No direct evidence is available indicating whether certain species do not require vitamin K or are able to effect a synthesis of the vitamin through the activity of some organ. At present, however, it is most likely that the difference noted is the result of difference in the absorption of antihemorrhagic substances as produced through the metabolic activity of the intestinal flora The longer intestinal tract in mammals is considered to favor such an absorption, as contrasted to the short colon of fowls. This assumption needs further qualifications.

The full consequence of the lack of vitamin K for the mammal organism was *first* revealed through the suggestion of *Quick* that the low prothrombin value and the hemorrhagic tendency noted in cases of obstructive jaundice might be caused by a lack of absorption of the vitamin due to the absence of bile in the intestines. *Numerous experimental and clinical investigations* have proved the validity of this suggestion Bile or bile acids of sufficient concentration must be present in the intestinal tract in order to obtain absorption of vitamin K These investigations are to be considered in detail in the subsequent chapter.

A faulty absorption of the vitamin followed by a *low* prothrombin level and hemorrhagic manifestations has also been observed in cases of sprue and other intestinal disorders.[62, 63]

References

1. Dam, H : Cholesterinstoffwechsel in Hühnereiern und Hühnchen, Biochem. Ztschr. 215: 475-492, 1929.
2. Dam, H : Über die Cholesterinsynthese im Tierkorper, Ibid 220: 158-163, 1930.
3 McFarlane, W D., Graham, W. R., Jr, and Richardson, Frederick. The Fat Soluble Vitamin Requirements of the Chick I. The Vitamin A and Vitamin D Content of Fish Meal and Meat Meal, Biochem J. 25: 358-366, 1931
4 Holst, W F, and Halbrook, E R A "Scurvy-Like" Disease in Chicks, Science 77: 354, 1933.
5 Dam, H.: Haemorrhages in Chicks Reared on Artificial Diets: A New Deficiency Disease, Nature 133: 909 910, 1934
6. Dam, *Henrik, and Schonheyder, Fritz A Deficiency Disease* in Chicks Resembling Scurvy, Biochem J. 28 1355-1359, 1934
7. Dam, H.: The Antihaemorrhagic Vitamin of the Chick; Occurrence and Chemical Nature, Nature 135 652 653, 1935.
8. Schönheyder, F The Antihaemorrhagic Vitamin of the Chick; Measurement and Biological Action, Ibid 135: 653, 1935.
9. Dam, Henrik: The Antihaemorrhagic Vitamin of the Chick, Biochem J 29 1273 1285, 1935
10. Halbrook, E R Quoted by Almquist, H. J., and Stokstad, E L. R.[11]
11. Almquist, H. J, and Stokstad, E L. R Dietary Haemorrhagic Disease in Chicks, Nature 136: 31, 1935.
12. Almquist, H. J., and Stokstad, E. L. R. *Hemorrhagic Chick Disease of Dietary Origin,* J. Biol Chem. 111: 105-113, 1935

13. Schönheyder, Fritz: The Quantitative Determination of Vitamin K, Biochem. J. 30: 890 896, 1936.
14. Dam, Henrik, Schönheyder, Fritz, and Tage-Hansen, Erik: Studies on the Mode of Action of Vitamin K, Ibid. 30: 1075-1079, 1936.
15. Dam, H., and Schönheyder, F.: Vitamin K, Nord. med. tidskr. 12: 1097-1105, 1936.
16. Almquist, H. J., and Stokstad, E. L. R.: Factors Influencing the Incidence of Dietary Hemorrhagic Disease in Chicks, J. Nutrition 12: 329 335, 1936.
17. Dam, Henrik, and Glavind, Johannes: Determination of Vitamin K by the Curative Blood-Clotting Method, Biochem. J. 32: 1018-1023, 1938.
18. Almquist, H. J., Mecchi, E., and Klose, A. A.: Estimation of the Antihaemorrhagic Vitamin, Ibid. 32: 1897-1903, 1938.
19. Almquist, H. J., and Klose, A. A.: Determination of the Anti-Haemorrhagic Vitamin, Ibid. 33: 1055 1060, 1939.
20. Thayer, S. A., McKee, R. W., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A.: Assay of Vitamin K Concentrates, Proc. Soc. Exper. Biol. & Med. 40: 478-481, 1939
21. Dam, Henrik, and Schönheyder, Fritz: The Occurrence and Chemical Nature of Vitamin K, Biochem. J. 30: 897-901, 1936.
22. Dam, Henrik, and Glavind, Johannes: Vitamin K in the Plant, Ibid. 32: 485-487, 1938
23. Almquist, H. J., Pentler, C. F., and Mecchi, E.: Synthesis of the Antihemorrhagic Vitamin by Bacteria, Proc. Soc. Exper. Biol. & Med. 38: 336-338, 1938
24. Almquist, H. J.: Purification of the Antihemorrhagic Vitamin, J. Biol. Chem. 114: 241 245, 1936
25. Almquist, H. J.: Purification of the Antihemorrhagic Vitamin by Distillation, Ibid. 115 589 591, 1936
26. Almquist, H. J.: Crystals With Vitamin K Potency, Nature 140: 25 26, 1937.
27. Dam, Henrik, and Lewis, Liese: The Chemical Concentration of Vitamin K, Biochem. J. 31: 17 21, 1937.
28. Almquist, H. J. Further Studies on the Antihemorrhagic Vitamin, J. Biol. Chem. 120: 635 640, 1937.
29. Dam, H, Geiger, A., Glavind, J, Karrer, P., Karrer, W., Rothschild, E, and Salomon, H: Isolierung des Vitamins K in hochgereinigter Form, Helvet. chim. acta. 22: 310-313, 1939
30. Almquist, H J, and Klose, A. A.. Color Reactions in Vitamin K Concentrates, J. Am Chem. Soc. 61: 1610 1611, 1939.
31. Fernholz, Erhard, Ansbacher, S., and Moore, Mildred L On the Color Reaction for Vitamin K, Ibid 61: 1613 1614, 1939.
32 Anderson, R J., and Newman, M. S The Chemistry of the Lipids of Tubercle Bacilli. XXXIV. Isolation of a pigment and of Anisic Acid From the Acetone-Soluble Fat of the Human Tubercle Bacillus, J Biol. Chem 101. 773 779, 1933.
33. Anderson, R. J, and Newman, M. S. The Chemistry of the Lipids of Tubercle Bacilli. XXXV. The Constitution of Phthiocol, the Pigment Isolated From the Human Tubercle Bacillus, Ibid 103. 197 201, 1933
34 Newman, M S, Crowder, J. A, and Anderson, R. J. The Chemistry of the Lipids of Tubercle Bacilli. XXXVIII. A New Synthesis of Phthiocol, the Pigment of the Human Tubercle Bacillus, Ibid 105 279 281, 1934
35 Almquist, H J., and Klose, A. A. The Antihemorrhagic Activity of Pure Synthetic Phthiocol, J. Am Chem. Soc 61. 1611, 1939
36 Almquist, H J, and Klose, A A The Antihemorrhagic Activity of Certain Naphthoquinones, Ibid. 61: 1923 1924, 1939.
37. Almquist, H. J., and Klose, A. A Synthetic and Natural Antihemorrhagic Compounds, Ibid. 61: 2557-2558, 1939
38. McKee, R. W, Binkley, S B, MacCorquodale, D W, Thayer, S A., and Doisy, E. A: The Isolation of Vitamins K_1 and K_2, Ibid. 61: 1295, 1939.
39 Binkley, S B, MacCorquodale, D. W, Cheney, L C., Thayer, S A., McKee, R W., and Doisy, E A. Derivatives of Vitamins K_1 and K_2, Ibid. 61: 1612 1613, 1939.

40. Thayer, S. A., Cheney, L. C., Binkley, S. B., MacCorquodale, D. W., and Doisy, E. A.: *Vitamin K Activity of Some Quinones*, Ibid. 61: 1932, 1939.

41. MacCorquodale, D. W., Binkley, S. B., Thayer, S. A., and Doisy, E. A.: On the Constitution of Vitamin K_1, Ibid. 61: 1928-1929, 1939.

42. MacCorquodale, D. W., Binkley, S. B., McKee, R. W., Thayer, S. A., and Doisy, E. A.: *Inactivation of Vitamin K by Light*, Proc. Soc. Exper. Biol. & Med. 40: 482-483, 1939.

43. Thayer, S. A., Binkley, S. B., MacCorquodale, D. W., Doisy, E. A., Emmett, A. D., Brown, R. A., and Bird, O. D.: Vitamin K *Potencies of Synthetic Compounds*, J. Am. Chem. Soc. 61: 2563, 1939.

44. Ansbacher, S., and Fernholz, Erhard: *Simple Compounds With Vitamin K Activity*, Ibid. 61: 1924-1925, 1939.

45. Binkley, S. B., Cheney, L. C., Holcomb, W. F., McKee, R. W., Thayer, S. A., MacCorquodale, D. W., and Doisy, E. A.: The Constitution and Synthesis of Vitamin K_1, Ibid. 61: 2558-2559, 1939.

46. Fieser, L. F., Bowen, D. M., Campbell, W. P., Fieser, Mary, Fry, E. M., Jones, R. N., Riegel, Byron, Schweitzer, C. E., and Smith, P. G.: Quinones Having Vitamin K Activity, Ibid. 61: 1925-1926, 1939.

47. Fieser, L. F., Bowen, D. M., Campbell, W. P., Fry, E. M., and Gates, M. D., Jr.: *Synthesis of Antihemorrhagic Compounds*, Ibid. 61: 1926-1927, 1939.

48. Fieser, L. F.: Synthesis of 2-Methyl-3-Phytyl-1,4-Naphthoquinone, Ibid. 61: 2559-2561, 1939.

49. Fieser, L. F.: Identity of Synthetic 2-Methyl-3-Phytyl-1,4-Naphthoquinone and Vitamin K_1, Ibid. 61: 2561, 1939.

50. Fieser, L. F., Campbell, W. P., Fry, E. M., and Gates, M. D., Jr.: Synthetic Approach to Vitamin K_1, Ibid. 61: 2559, 1939.

51. Tishler, M., and Sampson, W. L.: Antihemorrhagic Activity of Simple Compounds, Ibid. 61: 2563-2564, 1939.

52. Dam, Henrik, Schønheyder, Fritz, and Lewis, Lieve: The Requirement for Vitamin K of Some Different Species of Animals, Biochem. J. 31: 22-27, 1937.

53. Almquist, H. J., and Stokstad, E. L. R.: The Gizzard Factor of the Chick, J. Nutrition. 13: 339-350, 1937.

54. Dam, H., and Glavind, J.: Vitamin K i den menneskelige patologi, Ugesk. f. laeger. 100: 248-250, 1938.

55. Dam, Henrik, and Glavind, Johannes: Vitamin K in Human Pathology, Lancet 1: 720-721, 1938.

56. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: *A Quantitative Study on Blood Clotting*: Prothrombin Fluctuations Under Experimental Conditions, Am. J. Physiol. 114: 667-675, 1936.

57. Smith, H. P., Warner, E. D., and Brinkhous, K. M.: Prothrombin Deficiency and the Bleeding Tendency in Liver Injury (Chloroform Intoxication), J. Exper. Med. 66: 801-811, 1937.

58. Warner, E. D.: Plasma Prothrombin: Effect of Partial Hepatectomy, Ibid. 68: 831-835, 1938.

59. Dam, H., and Glavind, J.: Alimentary K Avitaminosis in Rats, Ztschr. f. Vitaminforsch. 9: 71-74, 1939.

60. Murphy, Rosemary: Possible Avitaminosis K Produced in Mice by Dietary Means, Science 89: 203-204, 1939.

61. Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B.: Additional Nutritional Factors Required by the Rat, J. Biol. Chem. 127: 23-42, 1939.

62. Holt, Hans: Fall av sprue behandlat med K-vitamin, Nord. Med. 3: 2428-2430, 1939.

63. Clark, R. L., Jr., Dixon, C. F., Butt, H. R., and Snell, A. M.: Deficiency of Prothrombin Associated With Various Intestinal Disorders: *Its Treatment With the Antihemorrhagic Vitamin (Vitamin K)*, Proc. Staff Meet., Mayo Clin. 14: 407-416, 1939.

CHAPTER XIII

THE HEMORRHAGIC TENDENCY IN DISEASES OF THE GALL BLADDER, BILE DUCTS, PANCREAS, OR LIVER

Introduction

"The benefit of the operation was shown in the immediate relief of pain, itching, nausea, vomiting and in the production of stools natural in color and odor. Death occurred, as it usually does in all such cases depending upon total occlusion of the bile ducts, from transudation of blood from the mucous surfaces, i e., from passive internal hemorrhage, the result of the poisonous effects of the biliary salts on the blood." This is Sims'[1] comment on the postoperative course of a woman upon whom he had operated in 1878, and upon whom one of the earliest cholecystostomies was performed for obstructive jaundice. We may infer that he and his contemporaries had a clear idea of the consequences of the postoperative complications encountered In fact, Baillie[2] had commented on this complication in 1795. They further conceived it to be secondary to changes in the blood effected by the obstructive process.

During the following sixty years the ever-widening field of surgery of the gall bladder and the bile ducts confronted an increasing number of surgeons with similar experiences,[3] and with the same disappointments as that of Sims; namely, an immediate relief of obstructive symptoms with heightened expectancy of continued improvement but for the sudden occurrence of postoperative hemorrhage reversing the entire picture

The seriousness of this complication of abdominal surgery about the end of the last and the beginning of this century is well pictured by the collective statistics of Petrén.[4-6] Based on reports, essentially from Scandinavian clinics, he found that postoperative "cholaemic" hemorrhages accounted for thirty of the 231 deaths which occurred after 1,891 operations on the gall bladder and the bile ducts Pallin[7] stated that among twenty-five patients operated upon for cancer of the bile ducts, fifteen of them succumbed from hemorrhage after operation

During the last half century or more, an enormous accumulation of data has been presented, covering all the various phases of this problem In spite of constant efforts the question has constituted a real medical puzzle up to very recent years

With the introduction of vitamin K therapy this problem must be considered solved, at least in its essential parts It seems therefore of little avail to present a review of the existing literature on this subject. Such reviews are to be found in several reports covering the field up to recent years[4-6, 8-12]

It may be of some interest, however, briefly to indicate the main lines along which the various investigative approaches have been directed

Two separate but closely linked problems are particularly prevalent to this development; namely, that concerning the etiology of the hemorrhages and the possibility of anticipating or predicting the potential hemorrhage.

As may be inferred from the introductory quotation from Sims, the idea was early prevalent that the pathologic processes giving rise to icterus in their turn resulted in certain hematologic changes of direct consequence for the subsequent hemorrhagic disposition. Particularly through Doyon's[15-27] experimental investigations of the incoagulability of the blood in relation to injury of the liver did the hypothesis arise that this hemorrhagic tendency was brought about through a low fibrinogen content of the blood. Through subsequent investigations, particularly of Whipple and associates,[28, 29] valuable information was uncovered It was found that small doses of hepatotoxins (chloroform, phosphorus, hydrazine) as well as tissue injury and inflammatory processes in general cause a stimulation of the particular activity of the liver that leads to production of fibrinogen A drop in fibrinogen was observed only after extensive necrosis of the liver had taken place. The findings satisfactorily explain the common observation, as frequently noted also in our cases, that the fibrinogen content of the blood, even at the time of most severe hemorrhage, has increased up to 800 to 1,100 mg. per 100 c.c. It is further to be recalled, as shown in the experimental part of the present work, that the coagulation time of the blood is not influenced by the content of fibrinogen before this concentration reaches a level approaching almost complete absence of fibrinogen.

Considerable efforts have been exercised in trying to prove the plausible detrimental effect on the blood constituents as caused by cholemia. This possibility was refuted by Morawitz and Bierich[30] in 1906 on the grounds that a concentration of bile salts necessary for the prevention of blood coagulation in vitro (0 5 to 1 per cent) exceeded many times that possible even in the most extremely jaundiced patients Among several others, Wangensteen[31] many years later refuted the idea of a true "cholemic" hemorrhage on the grounds that the concentration of bile acids in the blood is considerably lower than normally in the most severe cases of jaundice, in which the risk of hemorrhage was most pronounced

Many painstaking investigations have resulted from the supposition that retained bile pigments in the blood and the tissues may combine with the blood calcium and thereby deprive the blood of an essential component for its normal coagulation This assumption resulted in a therapy which for years was looked upon as promising Mayo Robson[32] in the middle nineties used preoperative and postoperative calcium therapy as a routine procedure in cases of jaundice. In 1930 Gunther and Greenberg,[33] in a review of the literature combined with careful personal investigations, concluded that "factors other than the amount of the available calcium must be sought to explain the abnormal bleeding phenomena seen in jaundiced patients"

Of no less surgical interest have been the efforts to find means by which to be able to predict the impending hemorrhage. From time to time certain

well-founded theoretical considerations have been advanced concerning the fallacy of associating phenomena, as observed through the coagulation of blood in vitro, with the admittedly quite separate problem of hemorrhage.[5, 33] According to this view it appears that efforts to prognosticate impending hemorrhage on the basis of hematologic investigation are doomed to failure and disappointment.

Nevertheless clinicians continued to accumulate data which intimated that the question was not to be closed on a purely theoretic basis As early as 1906 Morawitz and Bierich[30] maintained that in several cases of obstructive jaundice with hemorrhagic tendency was the coagulation time of the blood prolonged Numerous reports sounded the same optimistic note, although it must be admitted that the general methods employed on large clinical material frequently did not answer the general expectation to their prognosticating ability. Petrén,[6] on the basis of careful investigations, stated that the determination of the *coagulation time of the blood is of definite practical value in jaundiced cases*

Our own investigations along this line go back to 1932[34] A review was previously given of the findings and considerations leading to the conclusion that the test based on the plain recalcification of oxalated or citrated plasma represents a general test of blood coagulability and not a test for prothrombin. As such it was applied to a larger group, namely, ninety cases of obstructive jaundice (1932). It was found that the coagulation time of oxalated plasma in the jaundiced group as a whole was definitely prolonged in relation to a control group of 231 normal subjects, further that the coagulation time in bleeding cases was markedly prolonged in relation to the average normal. Observations at frequent intervals indicated the rapid changes in coagulability after the operation, indicating that reliance could not be placed on the findings of a normal coagulability before the operation. Increasing knowledge regarding the dependability and the limits of the test has not detracted from the conclusion of these observations. The modifications as required by the use of the photo-electric reading technique have not changed the principle A subsequent report[35] (1936) based on photo-electric observations consequently meant a direct substantiation and elaboration of our first findings

Working on the idea that the defect of coagulation in these cases was likely to be found in a prothrombin deficiency, Quick[36, 38] succeeded in transforming the test of recalcified plasma into a specific test for prothrombin by the addition of maximal concentrations of thromboplastin With this test Quick, Stanley-Brown, and Bancroft[37] found a diminished quantity of prothrombin in many of the cases with obstructive jaundice "*suggesting that the hemorrhagic tendency in jaundice is caused by a diminution of prothrombin.*" Several subsequent reports by other workers, particularly by Smith and his co-workers using their two-stage technique of prothrombin determination, have substantiated these findings. It need hardly be stressed that this development represented a most significant thrust toward the elucidation of the whole problem A *reduction of prothrombin to about one-half of the normal is con-*

sidered to bring the patient within the danger zone as far as bleeding is concerned, while actual hemorrhagic manifestations usually do not appear before the prothrombin is reduced to about 20 per cent of the normal quantity or less.

No less significance is to be attributed to the series of important reports subsequently appearing at short intervals.

Hawkins and Whipple[39] (1935) found in bile fistula dogs a gradual decline in the clotting time of recalcified plasma, *followed after a time by hemorrhagic* manifestations. This hemorrhagic tendency could be cured or entirely prevented by the administration of large daily amounts of beef bile. Hawkins and Brinkhous[40] (1936) determined the hematologic changes in biliary fistula dogs to result from depletion of prothrombin which could be raised to normal quantities by the administration of bile by mouth. After partial hepatectomy in rats Warner[41] (1938) found a pronounced drop in prothrombin during the first 24 hours with occasional occurrence of hemorrhagic manifestations. The prothrombin returned to normal values within the next 6 to 10 days; that is, somewhat earlier than the 3 weeks generally required for full regeneration of the liver

In a letter to the editor of the *Journal of the American Medical Association*, Quick[42] wrote (1937). "The prolonged restricted low fat diet and the poor absorption of fat soluble material due to the absence of bile in the intestines can conceivably so reduce the supply of vitamin K that the stored reserve is no longer adequate, whereupon the prothrombin begins to diminish. If this hypothesis is correct, vitamin K is therapeutically indicated. In fact it is not improbable that small amounts of powdered *alfalfa* and bile salts administered orally may perhaps *effectively prevent the postoperative hemorrhage* encountered in certain jaundiced patients" It seems to me only fitting to quote this communication It presents a well-founded hypothesis. Subsequent investigations have proved it most fruitful.

The first practical demonstration of the beneficial effect of vitamin K in the presented problem was given by Greaves and Schmidt[43] (1937). In bile fistula rats they found that administration of a crude extract of alfalfa and bile salts by mouth resulted in an increase in the prothrombin of the blood in these animals

In the beginning of 1938 came in close succession reports of independent investigations by Warner and co-workers,[44] by Butt and co-workers,[45] and by Dam and associates,[46, 47] conclusively demonstrating the beneficial effect of vitamin K in a clinical material. Extracts of vitamin K by mouth, together with bile salts, increased the lowered prothrombin content of the blood, prevented the occurrence of postoperative hemorrhages where such might have been expected on the basis of previous clinical experiences, and in most of the cases resulted in an arrest of hemorrhages.

An ever-increasing number of reports,[48-64] based on the use of extracts or *synthetic products* with antihemorrhagic activity, have substantiated the preventive and therapeutic value of vitamin K in the present group of diseases.

Clinical Investigation

The coincidence of the more general adoption of the test for prothrombin in hemorrhages of jaundiced cases and the introduction of vitamin K as a therapeutic antihemorrhagic agent to a certain extent may have interfered with a full clarification of variations of the prothrombin level after operation without vitamin K. This coincidence further may have obstructed a clear view of the effect of the antihemorrhagic treatment in these cases as practiced before the introduction of vitamin K. For this reason it has not been readily answered whether vitamin K in these cases is to be considered an important adjuvant or the very therapy.

These points are of direct practical importance. They are further of no less significance for a fuller understanding of the mechanism of the prophylactic and curative effect of vitamin K. The following investigations have been presented with these points in mind. They are considered of further practical significance through the findings that a satisfactory prognostication of the hemorrhages and the rapid variations of coagulability of the blood may be had by the employment of simple recalcification of the blood plasma without the use of thromboplastic material. Since 1932 this test has proved to us to be of definite practical value. It may be employed with advantage by others who may lack the opportunity to employ the admittedly more satisfactory but somewhat more complicated test for prothrombin

Material.—The present material consists of observations of the coagulability of the blood plasma of fifty cases of diseases of the gall bladder, the bile ducts, the pancreas, and the liver Five of these patients were not operated upon, while the remainder underwent surgical procedures of various types. The majority of the patients were observed at the Mayo Clinic on the surgical service of Waltman Walters, the others were observed at Surgical Department A, Rikshospitalet

The presentation of the available data has necessitated a convenient grouping of the cases Several approaches to a grouping have been tried, such as the nature and the duration of the primary disease, the type of operation performed, as well as the duration and intensity of the icterus On the basis of the available data, none of these approaches has yielded satisfactory results.

In the following a grouping has been undertaken on the basis of the degree of variation in the coagulability of the blood plasma following the operation The nonoperated group has been considered separately. In the former the cases more or less naturally fall into four different groups A sharp division between those cases belonging to Group 3 and Group 4 seems problematic in some instances, a point of minor consequence, however, for the final deductions

One point ought to be stressed. The cases investigated do not represent cases observed consecutively on the surgical services The investigation was aimed at an understanding of the prognosticating merit of the present test so far as postoperative hemorrhage is concerned, consequently, it was preferably applied in cases where such a complication was considered probable This fact

explains the rather large number of cases exhibiting clinical evidence of hemorrhagic tendency, and should not be taken to indicate the frequency of this complication on a regular surgical service.

As will be noted repeated observations during the postoperative course have not been possible in all the cases, particularly in Groups 3 and 4. Many of the latter cases have been going through a stormy postoperative course, necessitating the utmost consideration on the part of the attending assistant as to the reasonable comfort of the patient and a careful preservation of the available veins for necessary intravenous injections.

From a didactic viewpoint the type of grouping to be presented at first sight may appear disappointing, particularly to the surgeon, who *before* the operation has to decide upon the probable operative risk and act accordingly. To him it may appear a bit academic to group a case *after* the postoperative course has ended. On second thought, this survey may still serve his purpose as well as mine whose task it is to present the merits of the employed method and the justification of subsequent conclusions.

Results.—A recording of the observations of the coagulability of the plasma at various stages during the treatment of the patient is given in Tables XXVIII-XXXII. The tables are more or less self-explanatory.

Group 1 is made up of cases in which no appreciable change in the coagulability of the blood took place after the operation.

Group 2 comprises cases in which the blood exhibited a definite prolongation of the coagulation time following the operation, the coagulation time reaching its maximal about the end of the first postoperative week and returning to normal from or during the second week.

In one of the twenty cases included in these two groups a slight hemorrhage occurred from the region of the gall bladder at the end of the second postoperative week. Postoperative hemorrhage did not develop in any of the other cases

The cases in Group 3 as a whole are characterized by a continuation of the prolonged coagulation time into the second or third postoperative week or longer. In all these cases moderate to severe postoperative hemorrhage characterized the course following the operation Four of the patients died after operation from hepatic and renal insufficiency (Cases 24, 27, 29, 31). In one case death was indirectly related to the manifest hemorrhage through the plugging of the T-tube with subsequent cessation of the flow of bile (Case 27).

All the patients of Group 4 died following operation. All of them had clinical manifestations of hemorrhage, this being considered the main cause of death in seven of the nine patients of this group (see case reports). Taken as a whole, the group was further characterized by a gradual to rapid postoperative increase in the coagulation time of the blood, in most cases reaching extreme values

Of the five cases in the nonoperated group, operation was advised in one but not consented to by the patient (Case 49). Operation was considered contraindicated because of the extremely poor general condition of two patients

TABLE XXVIII

CHANGES IN THE COAGULABILITY OF BLOOD PLASMA IN GROUP 1 FOLLOWING OPERATIONS ON THE GALL BLADDER AND BILE DUCTS*

CASE	DIAGNOSIS AND OPERATION	COAGULATION TIME IN SECONDS														
		BEFORE OPERATION	DAYS AFTER OPERATION													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	…stomy and choledo- …omy for choledo- …sis	*115*				180	180		145				170			170
2	…stomy for cancer of the pancreas	165			195	165	120	155	210		145					150
3	…stomy and chole- …y for cholelithiasis …angitis; *empyema* …lder	–		165	175	150	170					165	185			
4	…tomy for chole- …intrahepatic jaun	150		155	130	215	145	185	140				160			
5	…duodenostomy for …tive stricture of the duct	–	170	170			*180*				170					130
6	…tomy, choledocho- …, and choledochos …cholelithiasis, cho …thiasis	–			185							200	165	175		
7	…stomy and choledo …for *cholelithiasis* …edocholithiasis	160				180		170			190					140
8	…strostomy for can …ead of the pancreas	160		150	210	170	170	210		190		160				

* …ve hemorrhage did not occur in any of these cases.

TABLE XXIX

CHANGES IN THE COAGULABILITY OF BLOOD PLASMA IN GROUP 2 FOLLOWING OPERATIONS ON THE GALL BLADDER AND BILE DUCTS*

CASE	DIAGNOSIS AND OPERATION	COAGULATION TIME IN SECONDS														
		BEFORE OPERATION	DAYS AFTER OPERATION													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
9	Cholecystostomy for cholelithiasis and cirrhosis of liver	180			305	255		200								
10	Cholecystostomy and choledochostomy for cholelithiasis, choledocholithiasis, and hemolytic icterus	255					360		220	270						
11	Cholecystostomy with removal of stones; choledochoduodenostomy for cholelithiasis, pancreatic obstruction of common duct (carcinoma ?)	300 240						660	420	280	230		210	230	215	200
12	Cholecystgastrostomy for obstructed common duct (tumor ampulla of Vater ?)	205		350	300	210	225							165		
13	Cholecystgastrostomy for carcinoma head of pancreas	270		280		210	360	270	175	170		210		180		
14	Cholecystgastrostomy for carcinoma head of pancreas	155		225	225	360	360				135		150			

15	Cholecystgastrostomy for tumor head of pancreas	230			360	360			205			175				
16	Cholecystostomy with removal of stones; choledochostomy for cholelithiasis, carcinoma head of pancreas	175		215	325	420	285	285	275		240		175			
	Second operation, choledochoduodenostomy	170		150	260	260	220	240								
17	Cholecystostomy with removal of stones for purulent chronic cholecystitis with stones	155		180		290	220	240	280	200	180	150	180	215	170	240
18	Transplantation external biliary fistula into stomach for stricture common duct (for first operation see Group 3, Case 34)	340						360				240				240
19	Cholecystectomy and choledochostomy for subacute cholecystitis and cholangitis	320 270 230 140				380			260							180
20	Choledochostomy for choledocholithiasis and cholangitis	270 (15")†			370 (50")				200 (22")						150 (25")	

*Postoperative hemorrhage occurred only in Case 9, in which slight oozing of blood from the gall bladder area occurred on the fourteenth postoperative day, *lasting for 3 days*

†The figures in parenthesis in Case 20 indicate prothrombin time in seconds.

TABL

CHANGES IN THE COAGULABILITY OF BLOOD PLASMA IN GROUP

CASE	OPERATION AND DIAGNOSIS	BEFORE OPERATION	COAGULATION TIM										
			DAYS AFTE										
			1	2	3	4	5	6	7	8	9	10	11
21	Cholecystostomy, choledocholithotomy, choledochostomy for cholelithiasis and chronic cholecystitis; hepatitis	270 250					495	525		500			
22	Reconstruction of common duct over rubber tube; stricture common duct												375
23	Cholecystostomy; extensive cirrhosis of liver												
24	Cholecystgastrostomy for cancer of body of pancreas	330					520						
25	Abdominal exploration; extensive cancer of the liver	210											
26	Abdominal exploration; cancer of common and hepatic ducts with complete obstruction	–		270				300					
27	Cholecystostomy and choledochostomy for cancer common duct	200		210	300		500	390		275	215		350
28	Cholecystostomy and choledochostomy for cholelithiasis, cholangitis and choledocholithiasis	270		270		360		540		315			300
29	Choledochoduodenostomy for cancer of the common duct	360 420 300			360			500		360		185	200
30	Choledochostomy for choledocholithiasis and cholangitis	–				400					450		
31	Cholecystgastrostomy for cancer of head of pancreas, biliary cirrhosis	780	None afterwards										
32	Cholecystostomy and choledochostomy for cholelithiasis and choledocholithiasis	–										420	
33	Exploratory cholecystostomy for cancer (?) common, hepatic, and cystic ducts	285 230			330					480		400	
34	Hepaticostomy, first stage operation for stricture of common duct (for obstruction after second operation see Case 18, Group 2)	650 840 250				480		550		540			410
35	Choledochostomy for choledocholithiasis and cancer of the papilla of Vater	720 290			340				470	520	630		720
36	Hepaticostomy for stricture of common duct	200 (16 seconds)					500 (75)	720 (90)	310 (35)	330 (40)		340 (30)	

*The figures in parentheses in Case 36 refer to simultaneous observations of the regular

XXX

Following Operations on the Gall Bladder and Bile Ducts

In seconds						
Operation						Remarks on postoperative course
12	13	14	15	16	17	
		390				210 seconds (22nd day): moderate bleeding through T tube during the middle part of second week
						From 7th to 15th day considerable bleeding from wound; also large hematoma abdominal wall
					345	Since 10th day *severe* bleeding from wound and dressed tube; *stools* contained blood; hemorrhage checked through repeated transfusions
						Considerable hemorrhage from wound about 8th day; *from* 15th day increasing uremia; death 24th day due to renal and hepatic insufficiency
390						Severe gastrointestinal bleeding beginning 2nd week, necessitating repeated transfusions, dismissed for transportation home 18th day, at which time hemorrhage had stopped
330		180				Moderate degree of bleeding from the wound on 12th and 13th days
		550				[illegible]
					320	
						Before operation ecchymosis arms, nose bleed, blood stained bile; from 1st day after operation bleeding gums, constant oozing from wound, hemorrhage checked at end of 2nd week; from 10th day rising urea, death 18th day from renal insufficiency
						From 7th day severe hemorrhage from wound, and gastrointestinal tract with melena and hematemesis, checked after repeated transfusions
						[illegible]
		210				Moderate oozing of blood through wound between 10th and 13th days, otherwise course uneventful; dismissed from hospital 17th day
	330					Before operation conjunctival, nasopharyngeal, and uterine hemorrhage, numerous ecchymotic spots over body, stormy postoperative course; hemorrhage from wound during 2nd week
	700		340		390	400 seconds (19th day), 240 seconds (36th day), very stormy course, severe bleeding from wound and through T tube from 6th day with temporary cessation flow of bile, followed by increasing icterus, temperature and urea; at end of 2nd week onset gradual slow improvement
245 (32 seconds)	*				170 (17 seconds)	From the 3rd day onset profuse bleeding from wound and through tube with drop in hemoglobin from 90 to 33 per cent, *poor* drainage of bile during bleeding, checked by repeated blood transfusions about 10th day

prothrombin time as expressed in seconds

(Cases 47 and 48) and not indicated in two (Cases 46 and 50). All of the cases exhibited changes in the coagulability of the plasma, in four of them of extreme degrees (Cases 46, 47, 48, and 49). In the two latter cases no clinical evidence of hemorrhage was present at the time of their hospitalization. Three patients evidenced symptoms of hemorrhage, and in one case it was the main cause of death (Case 46).

From the above findings it seems justifiable to conclude that *in a large number of cases in which diseases of the gall bladder, bile ducts, liver, and pancreas were present, certain hematologic changes take place, resulting in a moderate to most pronounced decrease in the coagulability of the plasma. The close*

TABLE XXXI

CHANGES IN THE COAGULABILITY OF BLOOD PLASMA IN GROUP 4 FOLLOWING OPERATIONS ON THE GALL BLADDER AND BILE DUCTS*

CASE	COAGULATION TIME IN SECONDS													
	BEFORE OPERATION†	DAYS AFTER OPERATION												
		1	2	3	4	5	6	7	8	9	10	11	12	LATER OBSERVATIONS
37	–									750				
38	330 420 190 340 190			270	370	540	1080							
39	–					1080						435		1080 (16th day)
40	240		480	810	900									
41	200 150			375		580			780				780	Ca 1080 (18th day) Ca 1100 (26th day)
42	300 230			400					420		460	500		
43	900 500			540					900					
44	510 360 230	600			1020				2000					
45	360			720										

*All patients succumbed following the operation. All of them had clinical manifestations of hemorrhage, this being the main cause of death in seven of them.

†The uppermost of the preoperative figures were obtained at beginning of preoperative treatment the lowermost, right before operation

parallelism as noted between the clinical manifestation of hemorrhage and changes in the coagulability of the blood in these cases indicate the hematologic basis of these hemorrhages This is a substantiation of conclusions made on previous occasions[34, 35] (1932, 1936) and represents a verification of a conception held by many early investigators

The findings in later years that these hematologic changes occur on the basis of a lowering of the prothrombin of the blood indirectly indicate that *the test employed here for determination of the coagulability of the plasma, when applied to this particular group of cases, represents a relative measure of the prothrombin content of the blood.* As is obvious from the preceding chapters this

TABLE XXXII

NONOPERATED GROUP

CASE	AGE, SEX	CLINICAL DIAGNOSIS	COAGULATION TIME (SECONDS)	CLINICAL COURSE
46	27 F	Chronic toxic hepatitis	1,980	Constant jaundice 1½ years, beginning two weeks after injection elsewhere of mercury preparation for skin eruption; serum bilirubin 12.5, increasing dur [illegible] per cen [illegible] (on ba [illegible] rhages [illegible] num, the subcutaneous tissues of the neck, to the lungs, to left ovary, to the ileum and bladder mucosa
47	[illegible]	[illegible]	[illegible]	[illegible]
48	[illegible]	[illegible] metastases to liver	[illegible]	[illegible] hemorrhage during 8 days in hospital
49	45 F	Biliary cirrhosis secondary to stone or stricture	780	[illegible] and choled- [illegible] rear intermit- [illegible] re upper ab- [illegible] vel of navel; [illegible]
50	[illegible]	[illegible]	[illegible]	[illegible] liver function fair; serum bilirubin 22 mg per cent, operation not indicated; during 10 days of medical regimen only moderate improvement in blood coagulability

TABLE XXXIII

THE LOCALIZATION OF POSTOPERATIVE HEMORRHAGE

SITE OF HEMORRHAGE	GROUP 3 (16 CASES)	GROUP 4 (9 CASES)	TOTAL (GROUPS 3 AND 4) (25 CASES)*
The operative wound, including accumulation of blood in abdominal cavity	13	8	21
Gastrointestinal hemorrhage	3	3	6
Bleeding through T-tube	5	2	7
Subcutaneous hemorrhages	3	3	6
Subconjunctival hemorrhage	1	–	1
Hemorrhage from kidney pelvis	1	–	1
Hemorrhage from oral cavity	2	–	2

*The figures of third column do not add up to the total number of patients as the hemorrhage in several instances was multilocular

is with the reservation that the patients do not have a marked thrombocytopenic purpura or hemophilia. When applied to any group of cases this test retains its unspecific character.

The figures and notes compiled in Tables XXVIII-XXXII answer directly the practical applicability of the test employed. *Of a total of thirty cases falling* within Groups 3 and 4 and the nonoperated group, twenty-eight cases gave clinical evidence of hemorrhage. The data indicate that *the test employed is of practical value when determining the potential hemorrhagic tendency in the present group.*

One point ought to be stressed in this connection. As late as the time of introduction of specific tests for quantities of prothrombin, doubt prevailed as to the possibility of forecasting a hemorrhagic tendency in this group on the basis of hematologic investigations. *In fact, the hematologic origin of the* hemorrhages was seriously doubted by many. This might have resulted from insufficient hematologic methods. However, with even the most satisfactory technique it will be readily understood, as demonstrated by the tables, that one single observation, for instance taken before the operation, can prognosticate little more concerning the postoperative course than, say, one single observation of the patient's temperature. This might seem pedantic to stress; but *the progressive nature of the changes in the coagulability of the blood and fortunately in most of them their regressive nature make it imperative to follow these changes by determination of the coagulability of the blood at short intervals.*

The frequent recording of changes in the coagulability of the blood is of direct consequence for the successful handling of these cases. When the progressive changes are halted and reversed, no hemorrhage may appear, as in Group 2. When this process of reversion takes place slowly and over a comparatively longer period due to the more pronounced changes in the coagulability, then hemorrhage may appear temporarily and cease without further consequence to the patient upon effective antihemorrhagic treatment Very rapid and pronounced progressive changes are usually followed by most serious hemorrhages. It is noted, as is apparent particularly in Group 4, that these pronounced changes may be present several days before the onset of the hemorrhage. It is inferred that *the degree and duration of the changes in the coagulability of the blood stand in direct relation to the degree of the subsequent hemorrhages.*

A couple of experiences included in the present material to some may appear to call for definite reservations in this respect, however. In Cases 48 and 49 there was no clinical evidence of hemorrhage during the patients' hospitalization in spite of the presence of pronounced reduction in the coagulability of the plasma. Their subsequent courses unfortunately are unknown to us. Neither are we in the position of knowing for how long these changes in the coagulability had been present. They might have been present for weeks Still, why no hemorrhage? Similar observations are familiar among hemophiliacs, many of whom may go about for weeks and months with the most extreme hematologic changes but with no manifest hemorrhages. Granted that this parallelism is of questionable value because of the different nature of the entities considered, more

convincing information is revealed by the present material itself. The above nonoperated group, when compared within its own entity, differs from the other four groups on one essential point: the cases of the former were not exposed to trauma, such as operation. The question of the traumatic factor causing the onset of hemorrhage is an essential point and will be considered separately.

It may be justified to infer that *in the present group of cases even the most pronounced decrease in the coagulability of the blood is not to be considered synonymous with clinical manifestation of hemorrhage. A traumatic factor of some kind must be considered one of the essential additional requirements for the creation of hemorrhage in these cases.* Further information concerning this point is to be furnished under next subheading.

Localization of the Postoperative Hemorrhages.—As listed in Table XXXIII the hemorrhages most frequently encountered in Groups 3 and 4 were those occurring in the wound, in most instances leading to an accumulation of blood in the abdominal cavity, and in two of the patients (Cases 39 and 44) directly contributing to the fatal outcome In most instances, as was to be expected, the hemorrhage was multilocular. From Table XXXIII it is obvious, however, that *the locus minoris resistentiae* as far as postoperative hemorrhage is concerned in this type of cases is represented by the operative wound.

The most serious type of postoperative hemorrhage encountered was that of the gastrointestinal tract, this occurred in three of the cases of Group 4 and was considered to have been the cause of death. It must be remembered, however, that in this group of cases it is frequently not easy to decide upon the main cause of death. Marked hepatic and renal insufficiency frequently preceded or was associated with the more marked changes in the blood coagulability.

A most distressing complication was that of hemorrhage occurring in the gall bladder and bile ducts that led to bloody secretions through the tube draining the common duct. This complication appears to have been insufficiently emphasized. Temporary bleeding through the T-tube may be of no further consequence. If unfortunately a clotting of blood should take place in the tube, resulting in a secondary obstruction to the flow of bile, a fairly satisfactory postoperative course may rapidly change into a grave picture This is illustrated by the following experience (Case 27).

CASE 27—On a woman, 55 years of age, cholecystostomy and choledochostomy had been performed for the relief of obstructive jaundice caused by cancer of the common duct. A marked decrease in the coagulability of the blood plasma was apparent during the first postoperative week, during the following days this showed satisfactory regression brought about by repeated transfusions of blood The postoperative course during the first few days was rather satisfactory, with a moderate fever subsiding after a few days. The secretion of bile had been satisfactory with quantities up to 550 c c. daily. On the fifth day a secretion of bile mixed with fresh blood was noted. On the seventh day the T-tube became completely clogged Complete cessation of bile drainage followed On the ninth day she suffered her first chill followed by several others and with rapid elevation of the temperature. The jaundice increased. Simultaneously a secondary marked prolongation of the coagulability of the blood was noted. Death occurred the fourteenth day after the operation. Necropsy revealed marked degeneration of the liver, which contained multiple abscesses

Similar experiences were noted in two other patients (Cases 44 and 45). In a fourth case the fatal outcome possibly was evaded through the accumulated bile finding its way around the clogged T-tube and escaping through the opened wound.

This complication may appear to be somewhat unexpected. After all, the concern of the surgeon during the postoperative course in these cases is that the blood may lose its property to clot normally, not that it will clot too rapidly. It is probable, however, that blood, markedly reduced in its coagulability, will coagulate when exposed to foreign material like that of the rubber tubing. This particularly will be the case when antihemorrhagic treatment like blood transfusions is pushed at the time of hemorrhage through the T-tube. Careful repeated irrigations of the T-tube with sterile solutions of citrate may be of some aid in keeping the tube patent when bleeding has occurred (Case 45).* To the surgeon I believe it would constitute an added sense of security if the T-tubes could be manufactured from some type of material that has a minimal coagulant effect on the blood.

Fortunately the other localizations of postoperative hemorrhage encountered in this material have been of less severe results to the patient; that is, in all except the two cases of cerebral hemorrhage The possibility of a purely coincidental cerebral hemorrhage in an older individual during the strain of the postoperative course must naturally be considered. One of the two patients with cerebral hemorrhage was a man, 64 years old (Case 41). Cerebral hemorrhages seem less likely to occur in younger individuals, such as the 27-year-old woman (Case 46) of the nonoperated group, or the woman aged 29 years, as reported by Snell and his co-workers.[83]

In considering peculiar coincidences, reference ought to be made to the findings in Case 42 At post-mortem examination the stomach was found to contain about 800 c c. of clotted and fluid blood. The jejunum, the ileum, and the colon were filled with clotted and liquid blood The intestinal mucosa throughout was markedly injected Microscopic examination of a suspicious area of the gastric mucosa revealed a gastric ulcer with erosion of the mucosa and undermining of the edges of the ulceration which extended well into the submucosa It is impossible to decide whether this massive gastrointestinal hemorrhage might have occurred even without markedly decreased coagulability of the blood, as was present in this case

At any rate, it appears from Case 44, in which the patient had had several severe episodes of gastric hemorrhage at a time when the coagulability of the blood might safely be assumed to have been fairly normal, that a persisting gastric ulcer does not necessarily start to bleed in the presence of markedly impaired coagulability. At post-mortem examination in her case a gastric ulcer was found at the lesser curvature. Whatever the relation between hemorrhage from gastrointestinal ulcerations and marked changes in coagulability of the blood, it must be kept in mind that such a coincidence, when present, constitutes

*If cessation of a previously satisfactory flow of bile occurs following clogging of the T-tube, particular attention must be directed to the probability of an accumulation of large quantities of bile having escaped around the T-tube into the abdominal cavity. If not relieved, such a complication invariably is fatal.

a grave problem. The mechanism of the arrest of hemorrhage is then pathologic and if not rapidly corrected may endanger the life of the patient. The history of the patient and information regarding the hematologic changes should make it possible to be prepared for any eventuality. Aside from the more common symptoms of pallor and drop in blood pressure in these cases, I have been impressed by the early occurrence of gas pains, abdominal distention, and impaired or interrupted intestinal peristalsis as early symptoms in cases of intra-abdominal or extra-abdominal accumulation of large quantities of blood. During the regular postoperative course these symptoms are by no means uncommon. In the presence of impending hemorrhage they may be rated, however, as of particular significance, especially when they occur after the immediate postoperative inactivity has passed.

The Hematologic Effect of the Operation.—From Tables XXVIII-XXXI it appears that operation undertaken in the cases presented was followed by a reduction in the coagulability of the blood. An exception to this rule are the cases of Group 1. The postoperative hematologic changes followed a pattern distinct enough to form the basis of a grouping as previously set forth.

What are the factor or factors active in bringing about these postoperative changes?

From the Tables XXVIII-XXXI it is seen that no essential difference exists between the various groups as far as the operative procedure is concerned, thus substantiating previous reports.[4, 5, 6] Identical types of operation are to be found in Groups 1 and 4. One of the patients in Group 4 died with hemorrhage after a simple exploratory laparotomy.

No essential difference existed between the postoperative hematologic changes and the anesthesia employed. In most of the cases ether, nitrous-oxide-oxygen anesthesia was given, frequently in combination with abdominal infiltration with novocain Only occasionally was spinal anesthesia used.

From these findings it is inferred that *the surgical procedure or the anesthesia do not per se determine the postoperative hematologic changes as recorded in the various groups of the present material.*

It is obvious from our findings that the progression of the hematologic changes is precipitated by an operation No such changes, however, were noted in Group 1. *It appears that in all cases an extrinsic factor as represented by the combination of the anesthesia and surgical intervention exerts its influence through an intrinsic factor of the patient. This latter factor determines the degree of the postoperative changes in the blood coagulability.*

The Nature of the Obstructing Lesion and the Intensity of the Jaundice

It may be of interest to search in the patient's clinical record to clarify whether or not some points of clinical nature can be correlated with the observed hematologic changes

In the individual case the pathologic condition present does not in itself indicate the presence or absence of changes in the blood coagulability. The various types of pathologic conditions are to be found in all the four groups

here considered. There appears to be a fairly even distribution of malignant conditions in the first two groups as compared to the last two groups. It has been maintained that hemorrhage is more likely to develop after operation for malignant lesions than for nonmalignant lesions. The selection of the present material makes it less suited for settling this point statistically. This question, however, is shortly to be taken up in a different connection.

Clinicians have maintained that the value of the serum bilirubin per se does not in the individual case indicate anything about the degree of changes in the blood coagulability.[69] In this connection reference will be made to a case of Group 2, in which the value for serum bilirubin outranges all others in any of the four groups (Case 14). This patient was found to have cancer of the head of the pancreas. The jaundice had progressed for three weeks. The value of the serum bilirubin was 60 mg. per 100 c.c. of blood at the time of his admission to the hospital. Considerable uncertainty was expressed as to the possibility of preparing this patient sufficiently for an operation which would be of grave risk to the patient. The normal coagulability of the blood at the beginning of his preoperative preparation of eleven days justified in our opinion some reason for optimism. At the time of operation the gall bladder was found to be thin walled and contained colorless bile The obstruction was found to be caused by a tumor of the head of the pancreas. The liver seemed to be in surprisingly good condition in view of the marked degree of icterus A regular cholecystgastrostomy was performed. The postoperative course was uneventful with no evidence of hemorrhage. The regressing changes in the blood coagulability took place after the fifth postoperative day. During the first five days after operation the value of the serum-bilirubin decreased from 48 on the day of operation to 25 mg. per 100 c.c. of blood on the fifth day; that is, during the time when the changes in blood coagulability are progressing This has been the regular experience in most of the cases belonging to Groups 2 and 3. It represents added evidence to the conception that *in the individual case the degree of jaundice stands in no direct relation to changes in blood coagulability.*

The duration of the jaundice has generally been considered to be a better guide to the clinician in this respect. In order to express this in satisfactory figures one would have to operate upon a considerably larger number of cases in which intermittent and permanent obstruction was present in each comparable group than is available by the present material.

A fair orientation may nevertheless be had by comparing the distribution in the various groups of the duration and intensity of the jaundice in cases in which complete obstruction was present caused by cancer of the bile ducts or the pancreas. It is then found that in Groups 3 and 4 the complete obstruction had lasted an average of 6 weeks with an average value of serum bilirubin at the time of admission of 23 mg., while the comparable figures for Groups 1 and 2 were 3 to 4 weeks and 26 mg serum bilirubin per 100 c.c. of serum. (It is readily understood that with the small number of cases compared a value of serum bilirubin of 60 mg in one of the cases of Group 2 brings the average value in

this group above that of Groups 3 and 4.) This finding is in general agreement with the investigations of Petrén who found that jaundice of about three weeks' duration rarely gave rise to postoperative hemorrhages, in contrast to cases in which the obstruction had been present for a longer time. It may be inferred that *the risk of an operation as far as postoperative hemorrhage is concerned is not directly determined by the intensity of the jaundice but is more closely related to the duration of the obstruction.* This finding further explains the general clinical impression[87] that in cases in which obstruction is caused by malignancy the patients more frequently have postoperative hemorrhage as compared to cases in which obstruction is intermittent.

Preoperative and Postoperative Treatment

The time factor of the progressing and regressing changes in blood coagulability as considered on previous pages must necessarily be viewed in direct connection with the medical regimen carried out before and after the operation. Otherwise it will be most difficult to reconcile these findings with certain previous reports in the literature. As an example the observation of Petrén may serve; that the danger of fatal postoperative hemorrhage in this type of cases is most imminent on the first and second postoperative days As will be recalled the postoperative hematologic changes noted in our cases were on the upgrade on the first and second postoperative days. Consequently, if this statement of Petrén is to be taken unconditionally, it seems definitely to refute the main thesis of this study, namely, that postoperative hemorrhages occur on a hematologic basis. Further investigations reveal this discrepancy to be only apparent. In this series of cases the hemorrhages, whether fatal or not, started at an average of 5 to 7 days after operation (for Groups 3 and 4 the average of 6 8 and 5 3 days, respectively), that is, at a time when the hematologic changes are at their maximal (Table XXXIV).

TABLE XXXIV

ACCOUNT OF TIME INTERVAL BETWEEN OPERATION AND THE BEGINNING OF THE POSTOPERATIVE HEMORRHAGE

Group 3		Group 4	
Case	Postoperative Days	Case	Postoperative Days
21	9	37	13
22	7	38	2
23	10	39	1
24	6	40	10
25	8	41	4
26	12	42	10
27	5	43	1
28	10	44	6
29	1	45	1
30	7		
31	4		
32	10		
33	10		
34	1		
35	6		
36	3		
Average	6 8		5 3

This comparison is also presented for another reason. I believe it forms a basis for a rough orientation as to the beneficial effect of the preoperative and postoperative treatment of later years. Referring to the collective statistics of Petrén, he found that 1.59 per cent of all patients operated upon succumbed from postoperative hemorrhage. Knowledge regarding the consequences of a carefully executed *preoperative and postoperative treatment*[23] was not available at the time Petrén's patients were operated upon. It seems only reasonable to presume that the liver of his patients at the time of operation might have presented a comparatively low content of glycogen. In that event surgical intervention would undoubtedly sooner exhaust the available "liver reserve," resulting in a rapid depletion of prothrombin and fatal hemorrhage at a much earlier postoperative date than was noted in our cases.

For obvious reasons, therefore, it seems desirable to state the regimen followed in the present cases:

In all of them the preoperative treatment has directly aimed at an increase in the glycogen reserve of the liver and its improved functional activity. The existing pathologic condition and the status of the liver determine in each case the limits of the beneficial effect of preoperative treatment, and consequently the time when the patient is considered ready for the operation. One has unfortunately no definite means by which to ascertain when this limit is reached. Purely on an empirical basis this limit is generally considered reached when the value for the serum bilirubin is stabilized on a minimum level, when the fever, if present, has subsided or become stationary, when the various tests of hepatic function are considered satisfactory under the circumstances, when the *tests for coagulability of the blood are normal or practically so, and when the* general condition of the patient has improved satisfactorily.

The routine preoperative treatment has consisted of a high intake of fluid, *a high carbohydrate diet, daily injections of hypertonic solutions of d-glucose* and saline, and blood transfusions as indicated

With this purely empiric regimen in mind it is of interest to note that, for the patients who *after* the completion of their postoperative course were considered of Groups 2 and 3, the duration of the preoperative treatment was averaging 8 and 12 days, respectively. Among several points, this may be taken to indicate that the experienced clinician from the total sum of the available data *before* the operation with almost exact accuracy can decide on the operative risk; further, that this risk to a great extent is relative to the regenerative power of the liver, as evidenced by its response to the preoperative treatment.

The average duration of the preoperative treatment in Group 4 was 10 days. This touches an interesting point. With the increasing seriousness of the clinical picture *one might expect a comparatively longer preoperative* period. This is apparently not so Due to the markedly insufficient hepatic function in many of these cases, the beneficial effect of an extension of the preoperative course may soon be counteracted by the progression of the pathologic process itself. Frequently, therefore, the surgeon is forced to operate

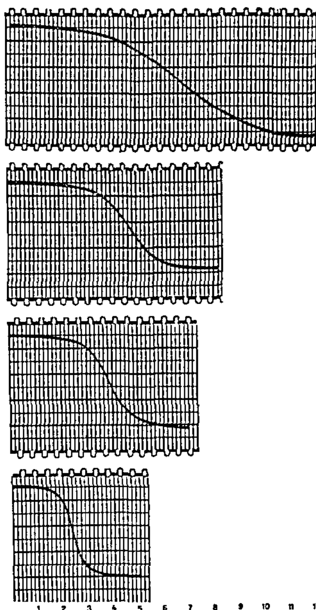


Fig 59—A series of four coagelgrams indicating the improvement of the coagulability of the blood during the preoperative course (Case 19) The figures below indicate minutes The upper coagelgram was obtained right after admission, eleven days before operation, the next, two, eight, and four days, respectively, and the lowest coagelgram, one day before the operation.

at a time when he is too well aware that the success of his surgical intervention is handicapped by a vicious circle of circumstances which, however, have to be interrupted in a last effort to avoid certain destruction of vital physiologic processes.

A further direct account of the effect of the preoperative treatment, as evidenced from changes in the coagulability of the blood plasma, is found in Tables XXVIII-XXXI. The lower figure for each case in the column for readings of the preoperative coagulation times generally indicates the reading obtained immediately before the operation.

In Fig. 59 an illustration is presented of changes in blood coagulability during the preoperative course as represented by a series of coagelgrams at various intervals after institution of the preoperative treatment (Case 19).

From all the data of consequence to the clinician during the preoperative course, it may be of interest separately to consider the preoperative changes in the blood coagulability and attempt a general formulation of what may be expected so far as the risk of postoperative hemorrhages is concerned:

1. A normal coagulability *before* the onset of the preoperative treatment indicates negligible risk of postoperative hemorrhage; it does not insure, however, against the generally seen decrease in coagulability during the first week after operation.

2. A moderate to a pronounced decrease in coagulability *before* the onset of preoperative treatment is practically always followed by a moderate to a pronounced decrease in coagulability during the first week after operation.

3. A moderate decrease in coagulability, rapidly corrected during the preoperative treatment, represents a negligible risk to postoperative hemorrhage.

4. A pronounced decrease in coagulability, only slowly or incompletely corrected during the preoperative treatment, constitutes an ominous sign. Not infrequently this is observed in connection with clinical manifestations, such as petechiae over the body, bleeding gums, subconjunctival hemorrhages, and so forth

The experiences here formulated may also be expressed thus· When bleeding occurs in these cases it is invariably associated with a decreased coagulability of the blood. The reverse is not necessarily true. The reduced coagulability signalizes an approach to a potential hemorrhage which primarily depends upon the degree of the hematologic changes present, secondarily upon extrinsic factors as expressed above, laying the ground for an exposure of the existing pathologic mechanism for arrest of hemorrhages.

On this basis a formulation of the main objective of the postoperative treatment as concerns postoperative hemorrhages appears clear *Its main objective is the maintenance of the coagulability of the blood so far as possible within the normal limits.*

In the present cases the nature of the postoperative treatment has not differed greatly from that of the preoperative treatment, except modifications as necessitated by the general reaction of the patient after operation.

It may be stated that the postoperative reaction has been in conformity with the grouping of the cases, the reaction having been moderate in Group 1, with generally rather stormy postoperative courses in Groups 3 and 4. The intensity of the postoperative treatment has varied accordingly, requiring the highest number of transfusions and intravenous injections of glucose and saline solutions in Groups 3 and 4

As indicated in Tables XXVIII-XXXI, in all groups except Group 1 is noted a moderate to a pronounced decrease in the coagulability of the blood plasma, and this in spite of careful postoperative treatment These changes have been in progression particularly during the first postoperative week; that is, during the time when the number of blood transfusions and injections of glucose and saline solutions has been highest. It is finally to be noted that the hematologic changes have been most marked in cases in which the severe postoperative reaction has necessitated the full mobilization of the available resources of the postoperative treatment A superficial reading of the tables may indicate the futility of the postoperative treatment as here carried out. That this is not so is readily demonstrated by the beneficial effect of the same type of treatment *before* operation in the very same cases in which postoperative progressive hematologic changes occurred in spite of the treatment. This indicates one main point. Without the benefit of the postoperative treatment these postoperative changes would have been considerably more marked, as evidenced from past experience with higher percentage and earlier occurrence of postoperative hemorrhages.

A reasonable explanation of the progressive postoperative changes must be sought in an increased demand for or increased destruction of prothrombin, a decreased production of prothrombin, or a combination of both

So far as increased destruction of prothrombin through fibrinous exudation in wounds is concerned, and by other regenerative processes in the body as suggested by Smith and co-workers,[48] no definite knowledge seems available at present, although such a possibility seems plausible.

Of greater consequence for an understanding of the requirements of an adequate postoperative treatment is the second possibility mentioned. In order to reach a logical exposure of this factor we have to pick out a series of indirect evidence and link it together as follows

At present, evidence clearly supports the contention that the liver is the essential organ for the production of prothrombin Other studies have presented significant information regarding the functional behavior of the liver after operation in the same type of cases as presented in this work. These studies are based on the finding that the ability of the liver to form bile acids might be utilized as a good indicator of at least one side of the functional activity of this organ Determination of bile acids has been performed on the blood or on the liver bile, with or without the injection of bile acids (Smyth and Whipple,[69] Bollman and Mann,[70] Snell and co-workers,[71] McGowan and associates[72]) Walters and his associates,[73, 74] investigating the constituents of the liver bile as excreted postoperatively through the T-tube, found an almost

complete absence of bile acids after long-standing complete obstruction of the common bile duct. After release of the obstruction, a formation of bile acids gradually took place, the increase being dependent upon the degree of the hepatic injury. Similar findings were subsequently made by Ravdin and associates[75] and Breusch and Johnston.[76] Gray and associates[77-79] undertook a similar study. Immediately after the operation they noted a decline in the concentration of bile acids in the hepatic bile, the concentration reaching a minimal level about the fourth to sixth day after operation. From this minimal concentration the increase in bile acids followed a certain pattern which could be fairly well correlated with the degree of the existing liver injury. In a case in which the liver was fairly normal the concentration of bile acids rose rapidly from the sixth day to about normal about three weeks after the operation. If the liver damage was more pronounced, this secondary rise in bile acids would take place over a considerably longer time and only with difficulty reach normal values much later. In cases of extremely severe damage to the hepatic parenchyma the ability of the liver to form bile acids was found to be almost exhausted. To these workers the investigations indicated that the study of bile acid concentrations in liver bile represents an approach to the evaluation of the functional activity of the liver of no less significance than the other laboratory methods available at present for the same purpose.

By comparison with the present work one point of the findings of Gray and his associates deserves particular attention. The minimal concentration of bile acids in the liver bile about the end of the first postoperative week coincides fairly accurately with the maximal level of the prolonged coagulation time of plasma as was noticeable in the uncomplicated cases of Group 2. The postoperative progressive and regressive changes in concentrations of bile acids occur further according to a pattern almost identical to the changes in coagulability of the blood and with parallel quantitative changes according to the degree of the liver damage. This certainly indicates more than a coincidence. Keeping in mind the fat solubility of vitamin K, the following suggestion presents itself: The relatively maximal deficiency of prothrombin in the blood at about the end of the first postoperative week is due to a minimal absorption of vitamin K as a consequence of a minimal concentration of fat-solvent constituents of the bile This reduces the whole problem to one of absorption of sufficient quantities of vitamin K. In this connection, of course, it would make no difference whether vitamin K actually was given in the diet or not. The intestinal canal under normal conditions is considered capable of sufficient production of vitamin K through the metabolic activity of the intestinal flora. If this be the whole story, administration of vitamin K and bile acids would result in the normal production of prothrombin Vitamin K under these circumstances would have to be considered as representing *the* antihemorrhagic therapy. This may not be quite true.

For the further understanding of the problem it may be permissible again to stress that the progressive and regressive changes as noted in the production of bile acids are in themselves an expression of changes in the functional activ-

ity of the liver during the postoperative course. Realizing that the liver is the essential organ of prothrombin production, it is suggestive that the progressive and regressive changes in the coagulability of the blood paralleling the similar changes in the formation of bile acids may indicate another expression of the functional activity of the liver. *If this postulate can be substantiated by further experimental investigations, the center of the problem would move from one of faulty, insufficient absorption to one concerning the functional inability of the liver to produce prothrombin.* Certain experiences by several investigators certainly substantiate this conception in extreme cases. I am referring to the inability of raising the prothrombin of the blood with adequate administration and absorption of vitamin K where the liver function is at its lowest ebb. To these extreme cases the saying may be applied that the mileage does not depend upon the gasoline but upon the motor. As suggested, it is likely that this also holds true for the less extreme cases

This brings us back again to a formulation of the meaning of the observed postoperative changes in blood coagulability. It is concluded that *in these cases an extrinsic factor as represented by the combination of the anesthesia and the surgical intervention exerts its influence through an intrinsic factor as represented by the functional activity of the liver. The latter determines the degree of the postoperative changes in the coagulability of the blood.* It goes without saying that this conclusion is not dependent upon controversial points of the nature of those raised above, as none of these patients received vitamin K therapy.

The practical consequence of this conception is evident. The preoperative and postoperative treatment as carried out in the present cases still retains, after the introduction of vitamin K, its preventive and therapeutic value in the field of surgery of the gall bladder and bile ducts. *It appears that the full success of vitamin K therapy in these cases will depend on a strict consideration of the maintenance of an adequate hepatic function in order to obtain the satisfactory response from the liver upon administration of vitamin K. In this respect the preoperative and postoperative treatment as employed in this series of cases serves its definite purpose.*

Summary

Determinations of the coagulability of the blood plasma have been undertaken in fifty cases of disease of the gall bladder, bile ducts, or the pancreas. Operation was performed in all but five cases.

According to the postoperative changes in blood coagulability the cases have been divided into several groups.

Group 1.—Eight cases. No postoperative change in blood coagulability. No postoperative hemorrhage

Group 2—Twelve cases Gradually increasing hypocoagulability of the blood during the first postoperative week, reaching a maximal about the fourth to the sixth postoperative day. Rapid return to normal during the second postoperative week. Slight postoperative hemorrhage occurred in only one case.

Group 3.—Sixteen cases. The postoperative hypocoagulability was found to be more pronounced as compared to the preceding group. The hypocoagulability continued during the second or third postoperative week with only slow return to normal. Postoperative hemorrhage occurred in all cases In five cases death occurred with signs of renal and hepatic insufficiency. In one of the cases hemorrhage was indirectly, in another case directly, related to the fatal issue.

Group 4.—Nine cases. All the patients died. A very rapid development was noted of a marked hypocoagulability of the blood during the first postoperative days. In all cases there occurred postoperative hemorrhages, in seven of them so severe as to be considered the main cause of death.

The Nonoperated Group.—Five cases. Excessive hypocoagulability of the blood was found in four cases, with moderate hypocoagulability in one case. *Massive hemorrhage with death occurred in one case, while there was only* moderate bleeding in two other cases.

Combining Groups 1 and 2 we find twenty cases with only one case among them exhibiting slight postoperative hemorrhage. Of the twenty-five cases of Groups 3 and 4 combined, all had clinical evidence of postoperative hemorrhage. It is concluded that *the present hematologic test as employed by these investigations, and undertaken at frequent intervals before and during the postoperative course, is of practical consequence for the execution of the preoperative and postoperative antihemorrhagic regimen.*

It is by now a well-established fact that the changes in the blood coagulability in the present cases are caused by changes in the concentration of prothrombin. It follows that *the test employed for determination of the plasma coagulability in these cases can be employed as a nonspecific measure of variations in the content of prothrombin*

The degree and the duration of the progressive changes in the blood coagulability determine the degree of the hemorrhagic disposition. The actual hemorrhagic manifestations appear as the result of the combination of progressive hematologic changes and traumatic factors, the latter associated with the operative procedure and the postoperative course.

According to their relative frequency, the localization of the postoperative hemorrhages is listed as follows: (1) hemorrhages from the wound, including hemorrhages into the abdominal cavity, (2) bleeding through the T-tube; (3) gastrointestinal hemorrhages, subcutaneous hemorrhages; (4) other types of hemorrhages.

The progressive changes in blood coagulability are not directly related to the type of operation, to the type of anesthesia, or to the intensity of the jaundice. It is, however, directly related to the duration of the complete obstruction of the bile ducts. A great risk of postoperative hemorrhage in the present cases was noted when the complete obstruction had lasted four weeks or longer.

A discussion has been presented concerning the rationale of the preoperative and postoperative regimen as carried out in the present cases The regimen

consisted mainly of frequent intravenous injections of hypertonic solutions of d-glucose, of blood transfusions and high carbohydrate diet. Vitamin K was not given.

The marked improvement in the coagulability of the blood as regularly seen following the preoperative regimen is interpreted as the result of improvement in the function of the liver. Where such a result is readily obtained by the preoperative management, the chances of postoperative hemorrhages are small. Where the preoperative improvement of the blood coagulability is very slow or incomplete, hemorrhages are impending.

The typical pattern of the postoperative progressive and regressive changes in the blood coagulability, as noted in the present cases, has been interpreted as being a true reflection of parallel postoperative variations in the functions of the liver.

The maximal degree of postoperative hypocoagulability coincided with the minimum output of bile acid, a coincidence reasonably explaining the prothrombin deficiency as resulting from a minimal absorption of vitamin K of endogenous origin.

The variations in the concentrations of bile acids, being in themselves an expression of the functional status of the liver, indicate that the progressive hypoprothrombinemia also results from an inability of the liver to form prothrombin in normal concentrations, even though vitamin K should be available in normal concentrations.

As a consequence of this latter contention it is suggested that the full success of vitamin K therapy in the present type of cases will only be attainable under strict attention to a preoperative and postoperative regimen capable of maintaining or improving the functional activity of the liver

Report of Cases

The following is a brief account of the history and the postoperative course of the patients belonging to Group 4 (Table XXXI) The case numbers correspond to those given in the table.

CASE 37.—A man, aged 63 years, registered at the Mayo Clinic July 17, 1936. He gave a history of having had malaria and typhoid fever in 1906 Otherwise he had been perfectly well until 9 months before this, when intermittent pain in the left of epigastrium had occurred 1 hour after meals. He had been treated elsewhere for a gastric ulcer The trouble had gradually progressed, and the man had lost weight, strength, and appetite Vomiting, gastrointestinal hemorrhage or jaundice had not developed.

On examination at the clinic the man appeared rather sick. A tender, fixed mass, about 10 cm. in diameter, was found in the right side of the epigastrium. Roentgenograms of the gastrointestinal tract gave negative results The stool was free of blood. The concentration of bilirubin was 1.8 mg per 100 c.c serum.

At operation on July 31, the liver was found to be filled with metastatic nodules Further exploration was not performed. Following the operation there was gradual onset of jaundice with rapid impairment of the general condition. From twelfth day there was continuous oozing of blood from the wound, increasing temperature, marked lowering of the blood pressure, increasing stupor, and death occurred on the fifteenth day after operation

Post-mortem examination revealed cancer of the bile ducts with metastases to the lungs, liver, spleen, gall bladder, pancreas, left suprarenal gland, thymus, and rectal shelf. Massive gastrointestinal hemorrhage was considered the main cause of death.

CASE 38.—A woman, aged 58 years, registered at the Clinic Sept. 2, 1936. Her health previously had been good. During the last 2 or 3 years she had had gaseous distress, and during the last 3 months had frequent attacks of gall bladder colic. After a most severe attack 7 weeks prior to examination at the clinic jaundice had developed which persisted and gradually increased. The stools were clay colored, and loss of weight was marked.

Examination revealed many purpuric spots over the woman's legs. A subconjunctival hemorrhage was noted in the right eye. The liver was moderately enlarged. The stools were negative for bile. The concentration of bilirubin was 20 mg. per 100 c.c serum. Coagulation time by the method of Lee and White was 18 minutes. Bleeding time by the method of Duke, 4 minutes and 35 seconds. Preoperative treatment for 2 weeks included blood transfusions twice.

Operation was performed Sept. 16. Palliative cholecystostomy was made, with removal of stones. Carcinoma of the gall bladder (primary?) and cholelithiasis were noted. A specimen for biopsy from the liver revealed induration which was an adenocarcinoma Grade 4.

The postoperative reaction was severe and immediate, but the patient responded well to blood transfusions. From the second day there was constant oozing of blood from the wound and through the dressed tube. From the fifth day there were severe chills and increasing temperature. The patient failed rapidly, and died the sixth day after operation.

Post-mortem examination revealed carcinoma of the gall bladder with obstruction of the bile ducts; metastases to the liver; hemorrhage into the gall bladder; hemorrhagic ascites and hydrothorax; edema of the lungs and infarct of the left hepatic lobe

CASE 39.—A woman, aged 51 years, registered at the Clinic June 11, 1935. In 1907 and 1913 cholecystostomies had been performed elsewhere with removal of stones. Upon recurrence, cholecystectomy had been performed in 1925. Choledochoduodenostomy for stricture of the common duct had been performed at the clinic in 1927. After this operation the woman had felt very well for 5 months, when nausea, vomiting, fever, and chills had begun. Since then she had had recurring, similar episodes associated with jaundice which cleared between attacks. Since the middle of November she had had persistent jaundice of moderate degree without pain

Examination revealed the woman was anemic. The concentration of hemoglobin was 9.8 gm. per 100 c c.; erythrocytes numbered 3,400,000 per cubic millimeter. The concentration of bilirubin was 7.9 mg. per 100 c c. serum Coagulation time (Lee and White) and bleeding time were normal. The woman received preoperative treatment for 13 days, including one blood transfusion

Operation on June 25 included dilatation of the choledochoduodenostomy, choledocholithotomy, choledochostomy for obstructed choledochoduodenostomy, choledocholithiasis. Cirrhosis of liver, Grade 4, was present.

Postoperatively, there was considerable oozing of blood from the wound for the first 4 days. On the fifth day there was a sudden change in condition with signs of massive gastrointestinal and intra-abdominal hemorrhage. The patient was in a very critical condition and was kept in an oxygen tent. During next 10 days she was given six blood [illegible] On the sixteenth day rate of respira- [illegible] chest developed, which was followed [illegible] th sudden drop in blood pressure The patient failed rapidly and death [illegible] h day after operation.

Post mortem examination revealed intraperitoneal hemorrhage and subphrenic and right perinephritic abscesses; cirrhosis of liver, Grade 3.

CASE 40.—A man, aged 62 years, registered at the Clinic May 13, 1935 During recent months he had had sharp epigastric pain after eating, which was associated with frequent belching and vomiting. During the last 4 weeks progressive jaundice, weakness, and anorexia had developed. There had been no rise in temperature. For two months he had been suffering from backache which disappeared after onset of jaundice.

Examination revealed that the liver edge was palpable and firm. The values for hemoglobin were normal. During the preoperative treatment of one week's duration the concentration of bilirubin increased from 15.2 to 20.7 mg. per 100 c.c. serum. The coagulation time by the method of Lee and White decreased from 16 to 9 minutes. *Bleeding* time was normal throughout.

At operation May 21 cholecystgastrostomy was made for tumor of the papilla of Vater. The postoperative course revealed that clinically the condition of patient seemed satisfactory during first few days. Because of rapid decrease in coagulability of the plasma the outlook, however, was considered grave and indicated the necessity of most active antihemorrhagic treatment. Blood transfusions were given daily, together with intravenous hypertonic solutions of glucose. In spite of this two large hematomas, localized over the region of both shoulder blades, appeared in the evening of the fourth day after operation. Next morning a hematoma had developed over the left thigh. From the sixth day there was constant oozing of blood from the wound. During the last two days the urinary output had been reduced with urea rising to 132 mg. and serum bilirubin to 26 mg per 100 c.c. of blood. The patient became stuporous, then comatose, and died the sixth day after operation.

Post mortem examination revealed the presence of a carcinoma of the common bile duct, intragastric and peritoneal hemorrhage, and bilateral hydrothorax.

CASE 41.—A man, aged 64 years, registered at the Clinic May 27, 1935. Slight anorexia had developed in October, 1934. From January, 1935, he had had painless jaundice, which had varied slightly in intensity Loss of weight was marked. Six weeks before admission he had had two short episodes of severe chills Examination revealed jaundice of moderate degree, and a slightly enlarged liver with some epigastric tenderness. The stools did not contain bile. The coagulation time by the method of Lee and White and the bleeding time were normal Preoperative treatment for 4 days included one blood transfusion.

Operation was performed on June 7 and included cholecystostomy, choledochostomy for chronic cholecystitis without stones and with perforating gall bladder. Suppurative hepatitis, choledochitis, and cirrhosis of the liver were observed. Following operation the T-tube drained well for the first 7 days, then only poorly. The man's general condition was fairly satisfactory for the first 3 weeks. From the twelfth day there had been considerable, constant oozing of blood from the wound. On eighteenth day bright red blood passed through the rectum and marked impairment of coagulability of the blood plasma had been noted at about this time On twenty-sixth day marked restlessness and paresis of the left leg and arm developed On twenty ninth day after a severe attack of coughing a subcutaneous hematoma suddenly appeared on the left side of the neck. During the last days before death, bleeding appeared from the mucous membranes of the mouth. The patient gradually failed and found swallowing very difficult toward end He died on the thirty-fourth day after operation

Post mortem examination revealed peritoneal, intestinal, and cerebral hemorrhages, chronic mitral endocarditis; cirrhosis of liver; bilateral hydrothorax

CASE 42—A man, aged 72 years, registered at the Clinic Jan 13, 1936 Prior to admission his health had been good. During the preceding two years he had had much gaseous distress, and three weeks before registration jaundice had developed, which had been painless at first, but after six days had been associated with attacks of severe, nonradiating dull epigastric pain, which had been relieved by heat The jaundice had been progressive with marked anorexia for the last few weeks, and the man had lost considerable weight.

Examination revealed that the liver edge was palpable about 3 cm. below the costal margin. A soft, regular, rounded mass was palpated below the liver edge. The man was moderately anemic. Serum bilirubin was 22.2 mg. per 100 c.c. of blood. The regularly employed coagulation time was 9 minutes, the bleeding time 1 minute. There was trace of bile in the duodenal fluid which also contained blood. The poor general condition of the patient made him a very great operative risk. In spite of this, operation was considered indicated as his only chance. Preoperative treatment carried out for 14 days included three blood transfusions.

At operation on Jan. 27, cholecystostomy was done with removal of stones, and choledochostomy for subacute gangrenous cholecystitis with stones. Hydrops of gall bladder, an obstructing lesion at the lower end of common duct (cancer ?), and cirrhosis of the liver were observed. Following operation drainage of bile through tube was negligible; however, copious bile was noted on the dressings. The concentration of serum bilirubin was only moderately reduced. The patient's condition remained rather poor throughout. On tenth day there was emesis of 400 c.c. of material resembling coffee grounds. The following day the man appeared in a state of shock, with increasing abdominal distention and pain. He failed rapidly in spite of blood transfusions and died 11 days after operation. Necropsy revealed the presence of cancer of the cystic duct with invasion of the gall bladder and common duct, gastric ulcer with massive gastrointestinal hemorrhage, and edema of the lungs.

CASE 43.—A man, aged 63 years, registered at the Clinic Aug. 31, 1936. His health previously had been good except for episodes of gout in 1935. About the middle of July painless jaundice, gradually progressing, had developed. He had had moderate nausea and marked loss of weight and strength (thirty-five pounds in 2 months). Examination revealed his color to be black-brown. There were numerous areas of ecchymosis all over his body. The edge of the liver reached the level of the umbilicus. The concentration of serum bilirubin was 50 mg. per 100 c.c. of blood.

During the 9 days of preoperative treatment the patient was given four blood transfusions with intravenous solutions of glucose twice daily. In spite of this and a high carbohydrate diet the general condition of the patient did not improve significantly nor was there any essential reduction of the jaundice. In the face of the desperate outlook we finally yielded to the request of the patient and he was operated upon on Sept. 8. Palliative cholecystgastrostomy was carried out for cancer of the head of the pancreas and hydrops of the gall bladder. Following operation there was constant oozing of blood from the wound from the time of operation, which later was coupled with massive gastrointestinal hemorrhage. The blood urea gradually increased to 306 mg. per 100 c.c. of blood, and the man died 9 days after operation. The findings at necropsy verified the presence of cancer of the pancreas as well as the massive gastrointestinal hemorrhage. The liver was of a Paris green color.

CASE 44.—A woman, aged 45 years, registered at Rikshospitalet (Surgical Department A) Nov. 13, 1937. Prior to this she had had good health up to 1930, when symptoms of bleeding gastric ulcer had been noted. During the following years the patient had been treated medically with satisfactory results. She sustained her first attack characteristic of gallstone colic in 1933; later these attacks occurred at frequent intervals but were not associated with jaundice. In 1935 dyspepsia recurred with hematemesis twice; this was followed by improvement on medical regimen. After a free interval gallstone colics recurred in October, 1936, associated with moderate jaundice. During the following year she had had repeated similar attacks, with jaundice and clay-colored stools; the last attack had occurred two weeks before admittance to the hospital.

Examination revealed a rather sick, markedly jaundiced woman, whose body had sustained numerous scratch marks. The liver was moderately enlarged. The serum color

according to method of Meulengracht was 1/120. The blood count and hemoglobin were normal and there was no bile in the stools Preoperative treatment for 9 days included two transfusions of blood.

On Nov. 22, 1937, cholecystectomy and choledochostomy were performed for cholelithiasis and choledocholithiasis. During the first week after operation the drainage of bile was satisfactory. The patient's condition was fairly satisfactory up to the sixth day when there was the onset of considerable abdominal pain, following impairment of intestinal peristalsis. Later in the same day there was the onset of profuse hemorrhage from the wound which necessitated packing. The subsequent state of collapse improved somewhat after emergency blood transfusion. At this time the secretion of bile was tinged with blood. On the eighth day the T-tube was clogged with clotted blood which necessitated persistent irrigation with sterile solutions of citrate. The secretion of bile completely ceased. From this point on the patient's condition became desperate, with constant oozing of blood through the wound, signs of increasing intra-abdominal accumulation of fluid, and increasing blood urea to 106 mg per 100 c c. of blood. Death occurred the twelfth day after operation. Post mortem examination revealed an accumulation of huge quantities of blood in the abdominal cavity. Anemic degeneration of the parenchymal abdominal organs was noted, particularly of the liver, which was Paris green in color A small gastric ulcer was present along the lesser curvature

CASE 45—A woman, aged 56 years, registered at the Rikshospitalet (Surgical Department A), June 1, 1938. She had been in good health up to 1929, when there was the sudden onset of fever and vomiting and after a few days moderate jaundice, which had lasted for 2 weeks. The woman had been admitted to a local hospital where her condition had been diagnosed as pernicious anemia. In 1930, in 1935, twice in 1937, and in 1938 she had received treatment in the same institution for the same disease. The anemia was satisfactorily controlled by liver therapy During her last stay there (1938) a second attack of intermittent fever had developed, which had had its onset every third or fourth day, simultaneously with the development of severe colic in the right upper abdominal quadrant. On May 9, 1938, she had been admitted to the Rikshospitalet (Medical Department B). She appeared rather sick and definite tenderness was present over the gall bladder, which was palpable. The blood picture was that of pernicious anemia. The percentage of hemoglobin was 76, and the number of erythrocytes 2,880,000 per cubic millimeter The stools were light brown. Treatment was immediately started for the anemia. During the following three weeks she had four attacks of coliclike pain in the right upper quadrant, the last two being very severe and associated with chills, fever, and jaundice. On June 1 cholecystectomy, choledochostomy, and choledocholithotomy were performed for subacute cholecystitis, choledocholithiasis, and cholangitis There was considerable oozing of blood from the liver bed during the operation. Following operation, there was steady oozing of blood from the wound. On the following day symptoms of accumulation of fluid in the abdominal cavity developed. Her general condition was very poor in spite of blood transfusions, and jaundice was increasing Only bloodstained secretions came through the T-tube Death occurred the third postoperative day, with the clinical picture characteristic of hepatic coma. Permission for post mortem examination was refused.

References

1 Sims, J. M: Remarks on Cholecystotomy in Dropsy of the Gall-Bladder, Brit. M. J. 1. 811 815, 1878
2 Baillie, Matthew: Quoted by Illingworth, C. F. W: Recent Developments in the Surgery of Obstructive Jaundice, With Special Reference to the Risk of Haemorrhage, Edinburgh M. J. 46. 762-780, 1939.
3 Mayo, W. J.: Surgery of the Hepatic and Common Bile Ducts, Brit. M. J 2: 7-8, 1923.
4 Petrén, Gustav. Ueber die postoperativen letal verlaufenden sog. cholamischen Blutungen, Beitr. z klin. Chir 110. 237-307, 1918.

5. Petrén, Gustav: Untersuchungen über die Blutgerinnung bei Ikterus, Ibid. 120: 501-538, 1920.
6. Petrén, Gustav: Untersuchungen über die Blutgerinnung bei Ikterus nebst einigen Worten über die sog. cholamischen Blutungen, Acta chir. Scandinav. 58: 433-522, 1925.
7. Pallin, Gustaf: Blood Transfusion in Cholaemia, Ibid. 55: 149-155, 1923.
8. Wangensteen, O. H.: The Haemorrhagic Diathesis of Obstructive Jaundice and Its Treatment, Ann. Surg. 88: 845-865, 1928.
9. Moss, Walter: Experimental Obstructive Jaundice; Its Effect on Fibrinogen and Coagulation of the Blood, Arch. Surg. 26: 1-19, 1933.
10. Carr, J. L., and Foote, F. S.: Progressive Obstructive Jaundice; Changes in Certain Elements of the Blood and Their Relation to Coagulation, Ibid. 29: 277-296, 1934.
11. Judd, E. S., Snell, A. M., and Hoerner, M. T.: Transfusion for Jaundiced Patients, J. A. M. A. 105: 1653-1658, 1935.
12. Gunther, Lewis, and Greenberg, D. M.: The Diffusible Calcium and the Proteins of the Blood Serum in Jaundice, Arch. Int. Med. 45: 983-1003, 1930.
13. Doyon, M., and Kareff, N.: Action de l'atropine sur la coagulabilité du sang, Compt. rend. Soc. de biol. 56: 192, 1904.
14. Doyon, M., and Kareff, N.: Action de l'atropine sur la coagulabilité du sang. Duree de la période d'incoagulabilité, Ibid. 56: 421-422, 1904.
15. Doyon, M., and Kareff, N.: Action de l'atropine sur la coagulabilité du sang. Role du foie, Ibid 56: 589-590, 1904.
16. Doyon, M., and Kareff, N.: Effet de l'ablation du foie sur la coagulabilité du sang, Ibid. 56: 612-613, 1904.
17. Doyon, M: Incoagulabilité du sang provoquée par le chloroforme; rôle du foie, Ibid. 58: 30-31, 1905
18. Doyon, M., Morel, A., and Billet. Altérations du foie provoquées par le chloroforme, Ibid. 58: 108-109, 1905.
19. Doyon, M., Morel, A., and Kareff, N.: Teneur en fibrinogène du sang rendu incoagulable par l'atropine, Ibid. 58: 428, 1905.
20. Doyon, M., and Kareff, N.: Action de l'atropine sur le foie. Coagulabilité du sang des veines sus-hépatiques, Ibid. 58: 444-445, 1905.
21. Doyon, M., Morel, A., and Kareff, N. Action du phosphore sur la coagulabilité du sang. Origine du fibrinogène, Ibid. 58: 493-494, 1905.
22. Doyon, M., and Billet, J.: Action élective du chloroforme sur le foie, Ibid. 58: 853-855, 1905.
23. Doyon, M, and Gautier, Cl.: Extirpation du foie et incoagulabilité du sang chez la grenouille, Ibid. 62: 521, 1907.
24. Doyon, M., and Gautier, Cl.: Ligature du tronc coeliaque et de l'artère mésentérique supérieure Modifications du sang, Ibid. 62: 650-651, 1907.
25. Doyon, M., and Gautier, Cl. Modifications de la coagulabilité du sang consécutive a l'anémie arterielle du foie. Action du sérum, Ibid. 63: 725, 1907.
26. Doyon, M., and Gautier, Cl.. Action comparée de l'atropine sur la coagulabilité du [illegible]
2[illegible]
2[illegible]
29. Fost[illegible]
30. Mor[illegible]

31. Wangensteen, O. H.: On the Significance of the Escape of Sterile Bile Into the Peritoneal Cavity, Ann. Surg. 84: 691 702, 1926.
32. Mayo Robson, A. W.: The Surgery of the Gall Bladder and Bile Ducts, With Brief Notes of 78 Cases, Brit. M. J. 1: 901-904, 1894.
33. Stegemann, Hermann: Zur Frage der Blutstillung in der Chirurgie, insbesondere zur Frage des spontanen Blutungsstillstandes, Beitr. z. klin. Chir. 127: 657-673, 1922.
34. Nygaard, K. K.: Coagulability of Blood Plasma: a Method of Determining Hemorrhagic Tendency of Jaundiced Patients, Proc. Staff Meet., Mayo Clin. 7: 691 696, 1932.
35. Nygaard, K. K., and Baldes, E. J.: Interpretation and Clinical Significance of Coagelgrams in Obstructive Jaundice, Ibid. 11: 705-709, 1936.
36. Quick, A. J.: The Prothrombin in Hemophilia and in Obstructive Jaundice, J. Biol. Chem. 109: lxxiii lxxiv, 1935.
37. Quick, A. J., Stanley Brown, Margaret, and Bancroft, F. W.: A Study of the Coagulation Defect in Hemophilia and in Jaundice, Am. J. M. Sc. 190: 501 511, 1935.
38 Quick, A. J., and Leu, Madeline: Quantitative Determination of Prothrombin, J. Biol. Chem. 119: lxxxi, 1937.
39. Hawkins, W. B., and Whipple, G. H.: Bile Fistulas and Related Abnormalities; Bleeding, Osteoporosis, Cholelithiasis and Duodenal Ulcers, J. Exper Med. 62. 599 620, 1935.
40. Hawkins, W. B, and Brinkhous, K. M.: Prothrombin Deficiency the Cause of Bleeding in Bile Fistula Dogs, Ibid 63: 795 801, 1936.
41. Warner, E. D.: Plasma Prothrombin; Effect of Partial Hepatectomy, Ibid. 68: 831-835, 1938
42. Quick, A. J.: Vitamin K, J. A. M. A. 109: 66, 1937.
43. Greaves, J. D, and Schmidt, C. L. A.. Nature of the Factor Concerned in Loss of Blood Coagulability of Bile Fistula Rats, Proc. Soc. Exper Biol & Med. 37: 43-45, 1937.
44. Warner, E. D, Brinkhous, K. M, and Smith, H. P. Bleeding Tendency of Obstructive Jaundice: *Prothrombin Deficiency and Dietary Factors*, Ibid. 37: 628 630, 1938.
45. Butt, H. R., Snell, A. M., and Osterberg, A. E.: The Use of Vitamin K and Bile in Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, Proc. Staff Meet., Mayo Clin. 13: 74 77, 1938.
46. Dam, H., and Glavind, J. Vitamin K i den menneskelige patologi, Ugesk. f. laeger. 100. 248 250, 1938.
47. Dam, Henrik, and Glavind, Johannes The Clotting Power of Human and Mammalian Blood in Relation to Vitamin K, Acta med. Scandinav. 96: 108 128, 1938.
48. Smith, H P., Warner, E. D., Brinkhous, K. M., and Seegers, W. H · Bleeding Tendency and Prothrombin Deficiency in Biliary Fistula Dogs; Effect of Feeding Bile and Vitamin K, J. Exper. Med. 67: 911 920, 1938
49. Butt, H. R., Snell, A M., and Osterberg, A. E.. The Preoperative and Postoperative Administration of Vitamin K to Patients Having Jaundice, J. A M A. 113· 383 389, 1939.
50. Brinkhous, K. M, Smith, H P., and Warner, E. D · Prothrombin Deficiency and the Bleeding Tendency in Obstructive Jaundice and in Biliary Fistula; Effect of Feeding Bile and Alfalfa (Vitamin K), Am J M Sc. 196: 50 57, 1938
51 Snell, A. M, Butt, H R, and Osterberg, A E Treatment of the Hemorrhagic Tendency in Jaundice, With Special Reference to Vitamin K, Am J Digest. Dis. & Nutrition 5. 590-595, 1938
52. Butt, H. R., Snell, A. M., and Osterberg, A. E Further Observations on the Use of Vitamin K in the Prevention and Control of the Hemorrhagic Diathesis in Cases of Jaundice, Proc. Staff Meet, Mayo Clin 13· 753 764, 1938
53. Greaves, J D.: The Nature of the Factor Which Is Concerned in Loss of Blood Coagulability of Bile Fistula and Jaundiced Rats, Am J Physiol. 125: 423-428, 1939.

54. Olson, P. F.: The Prothrombin Test and the Vitamin K Treatment for the Bleeding Tendency in the Jaundiced Patient, J. Iowa M. Soc. 29: 103-104, 1939.
55. Stewart, J. D.: Prothrombin Deficiency and the Effects of Vitamin K in Obstructive Jaundice and Biliary Fistula, Ann. Surg. 109: 588 595, 1939.
56. Caroli, J., Lavergne, H., Lavergne, B., and Bose, B.: Hémorrhagies des hépatiques; taux de prothrombine et vitamine K, Paris méd. 2: 75-86, 1939.
57. Townsend, S. R., and Mills, E. S.: The Use of Vitamin K and Bile Salts in the Prevention and Control of the Haemorrhagic Diathesis in Obstructive Jaundice (Preliminary Report), Canad. M. A. J. 41: 111-114, 1939.
58. Olson, K. B., and Menzel, Hildegard: The Bleeding Tendency in Obstructive Jaundice and Its Correction by Means of Vitamin K, Surgery 6: 206-220, 1939.
59. Stewart, J. D., and Rourke, G. Margaret: Prothrombin and Vitamin K Therapy, New England J. Med. 221: 403-407, 1939.
60. Frank, H. A., Hurwitz, A., and Seligman, A. M.: The Treatment of Hypoprothrombinemia With Synthetic Vitamin K; Report of 2 Cases, Ibid. 221: 975-977, 1939.
61. Macfie, J. M., Bacharach, A L, and Chance, M. R. A.: The Vitamin K Activity of 2-Methyl-1.4-Naphthoquinone and Its Clinical Use in Obstructive Jaundice, Brit. M. J. 2: 1220-1223, 1939
62. Butt, H. R, Snell, A. M., Osterberg, A. E., and Bollman, J. L.: Treatment of Hypoprothrombinemia Use of Various Synthetic Compounds Exhibiting Antihemorrhagic Activity (Vitamin K, Activity), Proc. Staff Meet., Mayo Clin. 15: 69 73, 1940.
63. Greene, C. H., and Hotz, Richard: Liver and Biliary Tract; a Review for 1938, Arch. Int. Med. 63: 778-808, 1939.
64. Ivy, A. C., and Gray, J. S: The Application of Recent Contributions in Basic Medical Sciences to Surgical Practice, Internat. Abstr Surg. in Surg., Gynec. & Obst. 69: 1-7, 1939.
65. Snell, A M., Vanzant, Frances R, and Judd, E. S: The Complications and Sequelae of Prolonged Obstructive Jaundice, M. Clin. North America 13: 1417-1438, 1930.
66. Arnsperger, Ludwig. Die chirurgische Bedeutung des Icterus. Zugleich ein Beitrag zur Pathologie und Chirurgie der tiefen Gallenwege, Beitr. z. klin. Chir. 48: 673-779, 1906.
67. Quénu, E.. Cancer des conduits biliares, Rev de chir., Paris 39: 462-481, 1909.
68. Walters, Waltman, Snell, A. M., and Clagett, O. T.: Surgery of the Biliary System. In Lewis, Dean: Practice of Surgery, Hagerstown, Md., 1929, W. F. Prior Company, Inc, vol. 7, pp 1-146.
69. Smyth, F. S., and Whipple, G H Bile Salt Metabolism. I. Influence of Chloroform and Phosphorus on Bile Fistula Dogs, J. Biol Chem. 59 623-636, 1924.
70 Bollman, J. L., and Mann, F. C. The Influence of the Liver in the Formation and Destruction of Bile Salt, Am J Physiol. 116: 214-224, 1936.
71. Snell, A. M., Greene, C. H, and Rowntree, L. G. Diseases of the Liver. VII. Further Studies in Experimental Obstructive Jaundice, Arch. Int. Med 40: 471-487, 1927.
72. McGowan, J. M., Bollman, J. L., and Mann, F. C. The Bile Acids in Icterus Produced by Toloylenediamine, J Pharmacol & Exper Therap. 58: 305-311, 1936.
73. Walters, Waltman, Greene, C H, and Fredrickson, C. H. The Composition of Bile Following the Relief of Biliary Obstruction, Report of a Series of Illustrative Cases, Ann. Surg. 91: 686-693, 1930.
74 Greene, C. H., Walters, Waltman, and Fredrickson, C. H.: The Composition of the Bile Following the Relief of Biliary Obstruction, J. Clin Investigation 9: 295 310, 1930.
75. Ravdin, I S, Johnston, C G, Riegel, C, and Wright, S. L.: A Study of Human Liver Bile After Release of Common Duct Obstruction, Ibid 12. 659-672, 1933.
76. Breusch, F, and Johnston, C G.: Zum Verschwinden und Wiedererscheinen der Gallensäuren in der Galle bei vorubergehendem Choledochusverschluss, Klin. Wchnschr. 13: 1856-1857, 1934.

77. Gray, H. K., and McGowan, J. M.: Liver Damage in Biliary Disease: Its Relation to the Concentration of Bile Acts in the Bile, Proc. Staff Meet., Mayo Clin. 12: 196-199, 1937.
78. Gray, H. K., Dutsch, W. L., and McGowan, J. M.: Effect of Biliary Operations on the Liver; Their Relation to the Concentration of Bile Acids in the Bile, Arch. Surg. 37: 609-618, 1938.
79. Gray, H. K., McGowan, J. M., Nettrour, W. S., and Bollman, J. L.: Hepatic Damage in Biliary Disease; Its Relation to the Concentration of Bile Acids in the Bile, Ibid. 37: 790-799, 1938.

CHAPTER XIV

HEMORRHAGIC DISEASE OF THE NEWBORN (HYPOTHROMBINEMIA HEMORRHAGICA NEONATORUM)

Among the pathologic conditions grouped under the term hemorrhagic diathesis one of the most poorly defined and obscure conditions has been that characterized by clinical manifestations of hemorrhage in the newborn. Several pathologic conditions have been noted to occur simultaneously with hemorrhages in the newborn (syphilis, sepsis, erythroblastosis, and so forth). The greater number of hemorrhages occur without coexisting disease. Minot[1,2] in 1852 stated that the hemorrhages appear in perfectly healthy infants and, if not fatal, appear to be of transitory, self-limiting nature. This point has later been stressed by Townsend[3] and a score of subsequent investigators.

In contrast to the other groups considered in this work this represents an extraordinary phenomenon, intimating that the condition may be viewed more as a transitory reaction than as a disease. On the other hand, death occurs in a certain percentage, either because of the localization of the hemorrhage to vital centers or due to its magnitude. This makes it difficult, to say the least, to consider the condition merely as an expression of a physiologic reaction

A review of older and more recent reports concerning this condition has been presented by Schloss and Commiskey,[4] by Lövegren,[5,6] by Salomonsen,[7] and by others and will not be repeated here.

During my work on the problems concerning the coagulability of blood plasma and its application to various clinical questions as previously presented in this work, the necessity was soon felt for extension of the hematologic investigations to include also those on newborn infants Through the encouragement and cooperation of Professor Anton Sunde it was possible to start this part of the investigation in January, 1939, at the obstetrical department of the University Clinic of Oslo.

At that time two important observations had been made. Brinkhous and his associates[8] had reported their finding of a hypoprothrombinemia of the newborn that lasted from the time of birth throughout the first year of life. Further, several reports had indicated the therapeutic effect of vitamin K in hemorrhagic conditions associated with obstructive jaundice.[9,10,11]

During the progress of this work, reports by Waddell and co-workers[12,13] indicated that vitamin K administered to newborn infants effected an increase in the quantity of prothrombin; similar findings were subsequently reported by Hellmann and co-workers.[14]

Simultaneous reports by Dam and associates[15] and by me[16] revealed the existence of an excessive hypoprothrombinemia in the newborn with hemor-

rhagic manifestations, and also that the prothrombin can be increased by vitamin K, the rise being followed by arrest of the bleeding tendency.

The present material consists of observations on 166 newborn infants delivered at the obstetrical department of the University Clinic, Oslo, between January, 1939, and January, 1940.

The determination of the prothrombin time has been performed according to the principle of Quick, with small technical modifications necessitated by the photo-electric reading technique. Quantitative estimations of prothrombin as expressed in units per cubic centimeter of genuine plasma have been performed according to the two-stage technique of Warren and co-workers. In order to prevent misunderstanding, let it again be stated that *the normal quantity of about 235 units prothrombin as reported by Warner and associates is comparable to about 330 to 360 units with the modifications employed in the present study.* When a two-stage technique of Warner and associates has been employed, it has been coupled with quantitative estimation of prothrombin based on a one-stage titration technique as performed either with serum or plasma. By either technique comparable results are obtained In the present report results are expressed on the basis of the two-stage technique in order to be able to present comparable results arrived at from principally identical technique.

In the majority of cases I personally have performed the puncture of the longitudinal sinus in order to ascertain that essential requirements for the successful collection of the blood specimen are duly considered. I believe this to be an essential point In no instances were the sinus punctures followed by any untoward reaction. All the investigations of the coagulability of the blood have been performed by me, excepting the coagulation time of whole blood which was undertaken by the laboratory technician according to the method of Sabrazè. The normal coagulation time according to the latter technique follows from the subsequent report.

Results

I. The Physiologic Variation in the Quantity of Prothrombin During the First Week of Life.—In Table XXXV an account is given of eighty-five observations of the prothrombin time of seventy-two normal newborn infants. Included in this table are forty-three observations previously published.[11]

The result of this investigation seems unequivocal. A normal prothrombin time is maintained during the first 10 hours after delivery. During the second half of the first day a definite prolongation of the prothrombin time is apparent. This prolongation is maintained during the following days From the sixth day of life (after 120 hours post partum) there is a return to normal prothrombin time, this subsequently being maintained.

As previously stressed, the prothrombin time is only an approximate expression of quantities of prothrombin Information regarding variations in the quantity of prothrombin had to be sought by quantitative methods. In Table XXXVI are listed units *of prothrombin in thirty-one normal newborn infants* at varying intervals post partum. The table is self-explanatory.

TABLE XXXV

THE PROTHROMBIN TIME AT VARYING INTERVALS POST PARTUM OF 72 NORMAL NEWBORN INFANTS (85 OBSERVATIONS)

PROTHROMBIN TIME (SECONDS)										
HOURS POST PARTUM										
0-10	10-[illegible]									
12	23									
12	27									
13	28									
13	30									
15	32									
16										
16		25	32	32		20				
16		27	33	32						
18		28	35	35						
19		30	35	40						
20		32	35	44						
20		33	40							
		40	45							
		40								
		40								

TABLE XXXVI

THE QUANTITY OF PROTHROMBIN AT VARYING INTERVALS POST PARTUM IN 31 NORMAL NEWBORN INFANTS

CASE	HOURS POST PARTUM	UNITS OF PROTHROMBIN PER C.C. GENUINE PLASMA
73	1	284
74	1½	284
75	6	272
76	9	213
77	9	264
78	10	266
79	14	125
80	19	105
81	30	81
82	32	71
83	35	103
84	48	110
85	50	113
86	51	73
87	53	134
88	55	95
89	55	103
90	55	110
91	62	80
92	65	108
93	66	92
94	67	107
95	67	89
96	102	135
97	130	102
98	144	195
99	148	128
100	190	195
101	216	187
102	218	355
103	29 days	360

It appears that *in the normal newborn infant there is a moderate hypoprothrombinemia during the first hours after delivery. From the second half of the first day there is a pronounced reduction in quantities of prothrombin. The low prothrombin level remains during the following days. From about the sixth to the seventh day post partum is apparent a secondary rise, which, however, does not bring the quantity of prothrombin up to the level characteristic for the first hours after birth.* Two observations (Cases 102 and 103) indicate normal values 218 hours and 29 days respectively after delivery. As these were isolated observations, opinion will be deferred as to whether or not this actually indicates that a normal prothrombin level is reached by the time noted. As will be recalled Warner and co-workers found a hypoprothrombinemia during the first year of life.

Among the forty-three observations in Table XXXVII (previously published[18]) the prothrombin values between the first and the sixth days of life all exceeded 20 seconds. These observations were undertaken between January and July, inclusive. Among the rest of the observations in Table XXXVII obtained between the months of August and December, inclusive, there were six observations with a prothrombin time during the comparable interval of 20

TABLE XXXVII

RECORD ON WHICH TO BASE COMPUTATION OF POSSIBLE DIFFERENCE IN PROTHROMBIN IN RELATION TO SEASONAL VARIATION*

GROUP OF CASE	PROTHROMBIN TIME (SECONDS)										
A											
	20		33 40 40	35 40 45 30	32 35 40 44	40	20				
Average	15 5	30	32.5	34 3	34 4	33 3	18	17.5	20	17 5	13
B	13 13 15 16 16 16 19 20	23 27	18 19 20 25 25 27 28 40 30	16 23 25 33 35 35	20 27 28 30	20 25	15 19 20	19 19	16 20	12 12	20
Average	16	25 5	25 8	28	26 3	22 5	18	19	18	12	20

*A, observations from January to July, inclusive, B, observations from August to December, 1939, inclusive

seconds or less. This intimated the possibility of a seasonal variation. Salomonsen[7] has noted such a variation in the coagulation time of capillary whole blood By a computation of the average prothrombin time for each day post partum in the two groups mentioned, it was found that the prothrombin time between the first and the sixth day post partum is relatively longer in the group examined during the first 7 months of the year, as compared to that examined during the

last 5 months of the year (Table XXXVII). This indicates that *under otherwise comparable conditions there is a relatively lower quantity of prothrombin in the blood of the newborn during the late winter and spring as compared to the autumn and early winter.* Whether comparable conditions in this connection will prove to include geographical considerations remains to be seen.

In seventeen mothers whose babies did not develop any hemorrhages, a quantitative estimation of prothrombin revealed a prothrombin content between 301 and 360 units per cubic centimeter genuine plasma. In four of these the blood specimen was obtained during the delivery, in six cases during the 2 weeks before, and in seven cases during the first week after delivery. It appears that *normally the blood of the mothers does not exhibit any quantitative deficiency of prothrombin.*

In seven cases the prothrombin time of blood from the navel cord was found to be within normal limits (Table XXXVIII). In three a quantitative estimation revealed a definite but moderate hypoprothrombinemia. Interestingly enough the quantity of prothrombin in specimens from two of the infants revealed a definitely higher value as compared to that of the navel cord The significance of these latter observations is reduced due to the fact that the specimens from the infants were not obtained before 1 to 1½ hours after delivery.

TABLE XXXVIII

THE PROTHROMBIN CONTENT OF BLOOD FROM THE NAVEL CORD

[illegible]	[illegible]	[illegible]	[illegible]
17	12		
23	13		
30	20		
	20	265	340* (2 hr. post partum)
	18	227	284 (1 hr. post partum)
73	18	176	

*The mother was given vitamin K 7 hours before delivery (50 mg 2-methyl-1,4-naphthoquinone).

In tentatively summarizing these findings it may be stated.

1. *The blood of the mothers usually contains normal quantities of prothrombin.*

2. *During the first 10 hours after delivery the blood of the newborn infants exhibits subnormal quantities of prothrombin. From this level the prothrombin rapidly decreases in quantity from the second half of the first day of life, remaining during the following days at a low level From the sixth day of life a secondary rise is noted in the prothrombin which reaches a level at least sufficient to bring about a normal prothrombin time from now on.*

3. *The transitory hypoprothrombinemia is at its maximum between 48 and 96 hours post partum.*

4. *This pattern of an initial reduction, a transitory hypoprothrombinemia and a secondary rise in quantities of prothrombin in the newborn is not subject to seasonal variations. It appears to express a physiologic postnatal reaction.*

5 *The transitory hypoprothrombinemia appears to be more pronounced during the months of late winter, spring, and early summer as compared to late summer, autumn, and early winter.*

6. *Blood from the navel cord appears to contain less prothrombin than that of the infant; however, it is sufficiently high to bring about a normal prothrombin time.*

Comment.—The present investigation substantiates the investigation of Owen and co-workers[17] that there is in the newborn a transitory hypoprothrombinemia during the first five days of life This explains the finding of Rodda,[18] which later was substantiated by Maurizio,[19] Sanford and associates,[20] and Salomonsen,[7] of a transitory prolongation of the coagulation time of whole blood during the same interval.

In two reports Quick and co-workers[21, 22] maintained that a profound fluctuation in the concentration of prothrombin was present during the first 48 hours, after which time the concentration usually returned to normal. This discrepancy in results, as intimated by Quick, cannot result from differences in technique. Part of this investigation employs the essential principle of Quick's technique. In view of subsequent findings it is not impossible that the results of Quick and his associates may be explainable on the basis of differences in the dietary regimen of the newborn.

From observations on ten normal newborn infants Waddell and Guerry[13] concluded that the prothrombin time during the first 5 days of life varied markedly from one day to another and from one individual to another The recorded prothrombin times varied between 40 and 600 seconds, in the latter instances without any evidences of hemorrhage. In the present series, at least, is noted a remarkably small variation from day to day and between the different individuals As will be brought out subsequently, great variations are to be noted only in cases with hemorrhages It is not improbable that the results of Waddell and co-workers were brought about by an inconstancy in the potency of the thromboplastic material

Hellman and Shettles[14] reported finding low quantities of prothrombin in the mothers With the two-stage technique of Warren and associates was found an average of 102 5 units per cubic centimeter of plasma, further, that this could be raised by administration to the mother of vitamin K. On both points the present findings are at variance with those of Hellman and his associates

2 **The Prothrombin in Relation to Hemorrhages of the Newborn.**—In Table XXXIX an account is given of the prothrombin time of seventeen newborn infants who exhibited various clinical manifestations of hemorrhage The blood samples were obtained as soon as possible after the clinical verification of the hemorrhages, and before any antihemorrhagic treatment was instituted In one case a specimen was incidentally obtained the day before onset of melena (Case 139)

A short review of the clinical course in the present cases is given at the end of this chapter.

It is of interest to note that in these cases the lowest prothrombin time *exceeds the longest prothrombin time as noted in normal newborn infants* and is evident from a comparison of Tables XXXIX and XXXV. In more than one-half of the cases of hemorrhage the prothrombin time markedly exceeded this physiologic value.

TABLE XXXIX

INVESTIGATIONS IN 17 NEWBORN INFANTS WHO EXHIBITED HEMORRHAGIC SYMPTOMS*

CASE	CLINICAL PICTURE	PROTHROMBIN TIME (SECONDS) HOURS POST PARTUM									
[illegible]	Melena		[illegible]	[illegible]							
140	Umbilical hemorrhage			55 T							
141	Umbilical hemorrhage			65†							
142	Cerebral hemorrhage			95 T							
143	Umbilical and subcutaneous hemorrhages			220 T							
		3 minutes after transfusion		55							
		Recurring hemorrhage				60					
		3 minutes after transfusion				20					
144	Subcutaneous hemorrhages				75 T				17		
145	Subcutaneous hemorrhages					75 T					10
		3 minutes after transfusion				35					
146	Fatal cerebral hemorrhage				150 T						
147	Cerebral hemorrhage		†§								
148	Cerebral hemorrhage (?), subcutaneous hemorrhages			55†	17						
149	Umbilical and subcutaneous hemorrhages			125†	15						
150	Twin 1, hemorrhage from ear canal			125†		16					
	Twin 2, umbilical hemorrhage			80†		20					
151	Umbilical hemorrhage				115†			25	19		
152	Fatal cerebral hemorrhage		135†								
153	Melena		†§								

[illegible] before treatment was instituted, a moderate to a pronounced coagula- [illegible]

§No sinus puncture performed because of serious condition; only capillary coagulation time of whole blood.

Significantly, the onset of the hemorrhage in the seventeen cases took place between the first and sixth day of life; that is, during the period of life which in normal infants is characterized by a physiologic hypoprothrombinemia

The coagulation time of whole blood before the commencement of treatment in these cases was found to be moderately to markedly prolonged (Tables XL and XLI). This finding substantiates and explains identical findings by Schloss and Commiskey,[4] Lövegren,[5, 6] and Salomonsen.[7]

How about the prothrombin level of the mothers of these infants? It is evident that by performing determinations of prothrombin of all mothers admitted to the obstetrical department one might finally have obtained a series of mothers whose offsprings developed hemorrhages. I have not been able to

carry out such an extensive investigation. Instead the blood of the mothers has been obtained for investigation immediately after verification of hemorrhages in the infants In none of these mothers was noted any prolongation of the prothrombin time. In five mothers quantitative estimation of the prothrombin revealed normal values. These investigations do not permit the conclusion that the prothrombin level is normal in mothers whose babies developed

TABLE XL

THE COAGULATION TIME OF WHOLE BLOOD BEFORE AND AFTER TRANSFUSION IN NEWBORN INFANTS WITH MANIFEST HEMORRHAGES

CASE*	COAGULATION TIME OF WHOLE BLOOD, MINUTES (CAPILLARY METHOD OF SABRAZE)							
	SOON AFTER ONSET OF HEMORRHAGE	HOURS AFTER BLOOD TRANSFUSION						
		6-24	24-48	48-72	72-96	96-120	120-144	144-168
139	10		4	3	4			
140	8	4	3	3	4			
142	10	6	5	7	7	5	4	4
143	13							
	11	5		4		4		
144	14	8		6	4			
145	8	4	3					
146	10							

*The case numbers correspond to those of Table XXXIX. In the present cases blood transfusion constituted the essential antihemorrhagic treatment.

TABLE XLI

THE COAGULATION TIME OF WHOLE BLOOD BEFORE AND AFTER ADMINISTRATION OF VITAMIN K IN NEWBORN INFANTS WITH MANIFEST HEMORRHAGES*

CASE†	COAGULATION TIME OF WHOLE BLOOD, MINUTES (CAPILLARY METHOD OF SABRAZE)									
	SOON AFTER ONSET OF	HOURS AFTER INSTITUTION OF VITAMIN K THERAPY								
149	8	5½	3		3	3½	3½	3½		
150 a	27	7	3½		2½	3	2½	3	3	
150 b	13	6	4	3½	4	5	4	3		
151	9		6½	4½	4	5	3½	3		
152	9½	7								
153	6½				3	3	3½	4½	4½	4½

*In the present cases administration of vitamin K constituted the essential antihemorrhagic treatment. No blood transfusions were given to these cases.

†The case numbers correspond to those of Table XXXIX and those of the case reports at end of chapter

hemorrhages A few days after delivery it is found to be within normal limits; what it might have been before the delivery cannot be judged by these investigations

The above findings indicate:

1. *The onset of hemorrhages in the newborn coincides in the majority of cases with the period of life characterized in the normal infant by a transitory hypoprothrombinemia.*

2. *Hemorrhages in the newborn are found to coincide with a hypoprothrombinemia exceeding that considered to be physiologic for this particular period in the normal infant. This does not prove, although it strongly indicates, that a marked hypoprothrombinemia is one of the essential factors in the pathogenesis of hemorrhages in the newborn.* I have suggested this condition be termed *hypothrombinemia hemorrhagica neonatorum.*

3. *With the reservations set forth above, the prothrombin content of the mothers in the present group appears not to differ essentially from that of the others.*

Comment.—Observations suggesting that hemorrhages in the newborn must be viewed in close connection with changes in the coagulability of the blood were recorded by early investigators many decades ago. In the reports of Minot,[1] previously mentioned, he refers to the incoagulability of the blood as observed in seven of eight cases in which the condition of the blood had been noted. Before his report Bowditch[23] (1850) emphasized the same peculiarity as did Weber[24] (1851). Lambert and co-workers[25] (1908) expressed the opinion that the cause of the hemorrhages was to be found in chemical changes in the blood. Similar conceptions were held by Schloss and Commiskey,[4] and by Lövegren,[5, 6] who noted a prolongation of the coagulation time of whole blood in cases of manifest hemorrhages, observations which subsequently have been substantiated by Beveridge[26] and Salomonsen.[7]

Of particular interest in this connection are the two reports by Whipple[27, 28] (1912 and 1913). In two fatal cases of melena Whipple investigated the quantity of thrombin which could be derived from the blood specimens. He found the thrombin to be definitely reduced in both instances. He concluded that the hemorrhages were caused by a lack of prothrombin. Of significance is his further statement: "It is obvious that the process leading to incoagulability of the blood was not congenital but developed, as the history would indicate, during the last two days of life." Credence in his conclusion and postulate may have been hampered by the fact that the observations utilized post-mortem blood, and in the first case, at least, by employing an investigative technique admitted by Whipple himself to be not beyond criticism I believe the present findings may have satisfactorily proved that Whipple's findings were correct. His conception that the hemorrhagic tendency is not congenital but acquired seems plausible, although for a somewhat different reason than held by him A closer scrutiny of this important question must await further observations of consequence in this problem.

The prevalence of these hemorrhages during the first week of life was commented upon by Minot as early as 1852 and possibly earlier by others. He stated specifically that the onset occurred at an average of 5½ days after delivery. Several subsequent investigators have elaborated on this point. Salomonsen, out of a total of sixty-six cases with manifest hemorrhages, noted the onset of bleeding in sixty-two to occur during the first 5 days of life

According to reports in the literature the frequency of hemorrhages in the newborn is less than 1 per cent At the University Clinic of Oslo, Salomonsen

collected sixty-six cases out of a total of 9,748 infants born between June, 1934, and May, 1939, inclusive, a frequency of 0.68 per cent. The highest frequency was noted during the months of winter and spring, the lowest between July and November, inclusive. This finding appears significant in view of the recorded seasonal variation in the transitory hypoprothrombinemia, a point also stressed by Salomonsen on the basis of the recorded seasonal variation in the coagulation time of whole blood Thus hemorrhages in the newborn on two different points can be viewed in significant relation to the normally occurring hypoprothrombinemia; first, through the prevalence of the hemorrhages during the period of physiologic hypoprothrombinemia in the individual case; second, through their highest frequency during the months of late winter and spring paralleling the relatively highest degree of physiologic hypoprothrombinemia.

In suggesting a term for the condition under consideration, I am well aware of the objections which may be raised. Thrombin as such does not circulate in the blood. When discussing in the present work quantities of prothrombin in the blood the term hypoprothrombinemia has been employed. The same term may conveniently be included in a designation of the condition under discussion. The terminology suggested above, however, implies, or is intended to imply, that the essential in the mechanism of the present hemorrhages is not only the quantity of prothrombin of the blood but primarily the quantity of thrombin formed at the site of the extravascular accumulation of blood If an insufficient quantity of thrombin is formed at this point, the mechanism of hemostasis is rendered deficient. The suggested terminology is felt to uphold this functional viewpoint. Further amplification on this point will shortly be presented.

3. **The Antihemorrhagic Effect of Blood Transfusions.**—In hemorrhage of the newborn blood transfusion has represented the standard treatment at the Obstetrical Department The beneficial effect of this procedure is so well established as to require no further comment.

When possible, citrated blood from the mother has invariably been employed and injected into the longitudinal sinus in relatively small quantities, from 15 to 20 c.c. at each transfusion

In cases of external hemorrhages that permitted visual observations of the bleeding, the common finding has been the almost immediate arrest of hemorrhages after completion of the transfusion. Its effect on the coagulability of the blood is recorded in Tables XXXIX and XL

It will be noted that the antihemorrhagic effect of blood transfusions is associated with a rapid improvement in the coagulability of the blood. What factor in the mother's blood brought about these changes? In these cases blood transfusions exert their effect through the quantity of prothrombin conveyed to the blood of the infant This is apparent from Table XXXIX. In two patients (Cases 143 and 145) the prothrombin time after transfusion on three different occasions was found to be markedly shortened.

Of particular interest is the observation in Case 143. The first transfusion was followed by cessation of the hemorrhages and a marked shortening of the

prothrombin time to within upper limits of the physiologic hypoprothrombinemia characteristic for that particular day of life. After two days, evidence of hemorrhage again appeared, necessitating a second transfusion. This was followed by a prompt reduction of the prothrombin time to within normal limits and with disappearance of the hemorrhagic tendency. The observation indicates that the transfused prothrombin is utilized fairly rapidly in the circulation of the infant. When this utilization occurs in the presence of a marked hypoprothrombinemia, the transfusion will exert its antihemorrhagic effect for a relatively short time; that is, until the prothrombin through its utilization is brought below a certain minimal level. Toward the end of the first week of life we have definite evidence that an increase in the formation of prothrombin occurs. An inherent production of prothrombin stems the consumption of the transfused prothrombin. Transfusions given in close proximity to this point may be expected to exert a hemorrhagic effect that lasts longer. It may also be expected that in order to give identical results the transfusions to be given early in the first week of life may safely be given in larger quantities. In addition the quantity ought to be graded according to the degree of the excessive hypoprothrombinemia, a deduction which seems evident if blood transfusions act through their content of prothrombin. This view is at variance with the conception of observers who maintain that the antihemorrhagic effect of transfusions is independent of the quantity of blood transfused. In the material of Salomonsen a second transfusion was indicated in nine cases of a total of thirty-seven requiring transfusions. From his tables it can be seen that in six of the nine cases the first transfusion was given during the first 3 days of life and in quantities averaging 17 c.c. citrated blood, a finding which seems to substantiate the above deduction.

It is inferred that *in hypothrombinemia hemorrhagica neonatorum blood transfusions represent a substitutional therapy As such, its ultimate antihemorrhagic effect is subject to gradation according to the degree of the hematologic abnormality and the quantity of the substituted principle*

Comment.—I should like to direct attention to the point that the satisfactory antihemorrhagic effect in this series was obtained with blood from the mothers of the bleeding infants. The series admittedly is small, but differs in no way from the beneficial effect of blood transfusions as noted in larger series This is an indirect evidence that the quantity of prothrombin of the mothers' blood must be considerably larger than that of the infants in order to produce the effect noted.

As to the necessity of preliminary agglutination tests it is known that agglutinins are rarely present at birth and during the first month of life. Occasionally the presence of iso-agglutinins and iso-hemolysins can be demonstrated in the newborn.[29, 30] Under ordinary circumstances this would indicate the advisability of preliminary matching of the blood of the donor and the recipient. In an emergency it seems permissible to eliminate the matching. In Salomonsen's series of forty-seven transfusions no preliminary agglutination test was ordinarily performed. In a group of 150 mothers and babies he found

the blood group to differ in thirty-five of them. In vitro agglutination did not occur in any of the cases, even when the mother's blood differed from that of the baby by belonging to Groups A, B or AB.

4. The Curative Effect of Vitamin K in Hypothrombinemia Hemorrhagica Neonatorum.—On the basis of the remarkable effect of vitamin K in raising *the hypoprothrombinemia in cases of obstructive jaundice and following the* demonstration of the excessive hypoprothrombinemia in cases of hemorrhages of the newborn, it was suggestive to try vitamin K in the latter group of cases.

Because of the satisfactory results obtained by blood transfusions it was deemed advisable to reserve the first trial with vitamin K for a case in which little would be risked should the vitamin prove ineffective. This opportunity presented itself with the following case: In a male infant delivered by version and extraction by forceps to the aftercoming head (transverse position) a cerebral hemorrhage developed (Case 147). Repeated attacks of generalized convulsions and deep cyanosis were so severe as to exclude almost any efforts at therapy Vitamin K was given in large doses, 50,000 units (Dam) were injected intramuscularly and an identical dose was given by mouth. Contrary to expectations the infant survived and a rapid and marked reduction in the coagulation time of capillary whole blood was noted. This experience furnished the necessary confidence in further trials without the aid of blood transfusions, which necessarily would have rendered observations inconclusive.

Up to the end of 1939 ten cases of hypothrombinemia hemorrhagica neonatorum have been treated with vitamin K at the University Clinic. Five other infants delivered at other local institutions and the cases not included here, have yielded essentially identical information The subsequent changes in the prothrombin time and the coagulation time of capillary whole blood are recorded in Tables XXXIX and XLI

During a time when there has been a rapid development in more potent and satisfactory preparations of extracts of vitamin K, as well as synthetic products, a good many preparations have been tried. It has been gratifying to note that all have exerted a satisfactory rise in the hypoprothrombinemia where such has existed

Of the ten cases of this series four of them were given a highly purified extract of vitamin K furnished by Dam (Cases 138, 141, 147, and 148). I have aimed at the administration of an initially large dosage, the rationale of this being obvious. In the four cases the purified extract (50,000 units [Dam]) was injected intramuscularly and a similar dose was given by mouth during the next few hours as the oily nature of the extract was not well tolerated by the infants when given undiluted by mouth A total dosage of 100,000 to 150,000 units admittedly represents a large dose of vitamin K per kilogram of body weight No untoward effects were noted

In the other six cases a synthetic preparation was used, namely, 2-methyl-1,4-naphthoquinone in quantities of 5 to 10 mg intramuscularly simultaneously with 5 mg by mouth These preparations have been taken by mouth without producing any vomiting in the infants Likewise the intramuscular injections

have been greatly facilitated because of the lower viscosity of the preparations as compared to the oily extracts before mentioned. The combination of the administration of the preparation by intramuscular injection as well as by mouth appears to be of consequence in order to ensure both a rapid and simultaneously a prolonged vitamin K effect. The vitamin was noted to be absorbed rapidly when given by mouth.

As may be seen in Table XXXIX, the excessive hypoprothrombinemia present in cases of manifest bleeding is corrected within 24 hours after administration of vitamin K. From a practical point it seems of little consequence whether the vitamin therapy is followed by an increase to entirely normal values, so long as it is able to bring the prothrombin time within normal limits as is shown to be the case. *In other words, it is demonstrated that a sufficient quantity of vitamin K corrects not only the excessive hypoprothrombinemia but also the physiologic hypoprothrombinemia characteristic for this stage of life. Most significantly, there is a simultaneous cessation of hemorrhages where such a visual observation is possible. The observations that an excessive hypoprothrombinemia is invariably found in cases of hemorrhages of the nature indicated, further that cessation of the hemorrhage coincides with a simultaneous rise in the quantity of prothrombin, indicate the important role played by this hematologic factor in the pathogenesis of hemorrhages in the newborn.*

Comment.—It will be noted from Table XLI that in several of these cases the coagulation time of capillary whole blood before onset of the antihemorrhagic treatment is considerably more prolonged than in those in which blood transfusion was given. In spite of this, however, the coagulation time on subsequent days for the group treated with vitamin K remains remarkably constant within the normal limits, and more so as compared with the group to which transfusions were given The two groups are too small for consequential comparison The figures, however, appear to intimate that blood transfusions exert a less profound and less permanent correction of the abnormal coagulability as compared with the group treated with vitamin K. The necessity of a second transfusion in certain cases may prove to point in the same direction.

As evident from Table XLI, the changes in the coagulation time of whole blood after medication with vitamin K is apparent to a strong degree within 3 to 7 hours. *I believe this is a point of some significance.* It brings up, among other things, the question whether or not vitamin K medication in the present condition is going to replace completely the previously employed blood transfusions. I personally believe it is going to do so in the majority of cases. It is possible that under certain circumstances the hemorrhages, when detected, may have been so excessive or so violent at the time as to necessitate an emergency blood transfusion in order to assure rapid arrest of the hemorrhagic tendency as well as a correction of the lowered blood volume. Fortunately in the present series no such cases have been encountered and they may be less likely to occur in the hospital where infants are under a different regimen and constant observation as compared to infants delivered at home

In one of the cases (152) death following cerebral hemorrhage occurred in spite of the early institution of vitamin K medication. The definite lag period of the efficacy of the vitamin therapy brings in the question whether a transfusion might have meant a lifesaving procedure through its immediate antihemorrhagic effect. If this ever were to be made a medicolegal problem, I do not know how it would be answered. In several cases of similar type the infants have succumbed in spite of early transfusions A parallel case in which transfusions were given and in which death resulted is that of Case 146.

In the other two cases in which cerebral hemorrhage was not fatal and the infants were treated with vitamin K, it was at any rate a gratifying experience to note the gentleness with which vitamin K therapy could be instituted as compared to the straining and crying that is unavoidably associated with blood transfusions of infants

With the introduction of synthetic preparations with great potency of raising the prothrombin level, it is to be expected that these preparations may gradually replace the more expensive purified extracts of vitamin K. In the present series satisfactory results were obtained with a single initial dose of a maximum of 15 mg. of the naphthoquinone. In one normal case an intended injection of 20 mg of the preparation was through misunderstanding repeated within 1 hour, without the appearance of any subsequent untoward effects. Its water solubility is very limited; when used in the present group, however, it was not necessary to administer it with bile salts as such are freely available in the intestines of normal infants and assure rapid absorption.

5. Prevention of the Development of Physiologic Hypoprothrombinemia Neonatorum Through Vitamin K.—In view of the striking curative effect of vitamin K in hypothrombinemia hemorrhagica neonatorum coupled with evidence that the excessive hypoprothrombinemia may be a postnatal phenomenon, a reasonable expectation is that this condition might effectively be prevented through the administration of vitamin K. As is to be subsequently considered, the etiology of these hemorrhages is a problem more complicated than may appear on first sight from the preceding exposé From the above findings the excessive hypoprothrombinemia encountered appears, however, to be a significant link in the process resulting in hemorrhages, which indicates that we are in possession of a good theoretical basis at least for the expectation stated. I believe the proof of this contention will eventually emerge from a large amount of clinical material collected over a long period of time.

The present investigations have aimed at the formation of a basis for such a preventive treatment as well as an orientation into various approaches which may be closely considered before such a preventive measure can be properly instituted.

A. Postnatal Administration of Vitamin K to the Infant.—To a group of twenty-five normal infants vitamin K was administered either immediately after birth or at varying intervals post partum (Table XLII). The preparation employed was either a purified extract of vitamin K or a synthetic product. In either instance the subsequent results appear to be identical.

TABLE XLII

THE PROTHROMBIN CONTENT OF NORMAL NEWBORN INFANTS AT VARYING INTERVALS POST PARTUM AFTER ADMINISTRATION OF VITAMIN K TO NEWBORN INFANTS

CASE	VITAMIN K ADMINISTRATION	PROTHROMBIN TIME (SECONDS) FIGURES IN PARENTHESES INDICATE UNITS PROTHROMBIN PER C.C. PLASMA						
112	50,000 Dam units intramuscularly immediately after delivery					19		
	post partum							
77	Naphthoquinone, 5 mg per os and 5 mg. intramuscularly 10 hours post partum	14* (264)			13 (310)			
78	Naphthoquinone, 10 mg intramuscularly 10 hours post partum	14* (266)						12 (266)
							19	
					15 54)			
					55*		19	
					12*	17		
					7*		13	
							25* (135)	15 (291)

The findings indicate that *administration of vitamin K to the normal infant immediately after delivery results in the prevention of the physiologic prolongation of the prothrombin time otherwise subsequently noted. If the medication is postponed until after prolongation has developed it is rapidly brought within normal limits.*

B. *Postnatal Administration of Vitamin K to the Mother.*—In a small series of cases identical results were obtained when vitamin K was administered, after delivery, to the mothers only (Table XLIII).

TABLE XLIII

THE PROTHROMBIN CONTENT OF NORMAL NEWBORN INFANTS AT VARYING INTERVALS POST PARTUM FOLLOWING ADMINISTRATION OF VITAMIN K TO THE MOTHERS AFTER DELIVERY

CASE	VITAMIN K ADMINISTRATION (2 METHYL-1,4-NAPHTHOQUINONE)	PROTHROMBIN TIME (SECONDS) FIGURES IN PARENTHESES INDICATE UNITS PROTHROMBIN PER C.C. PLASMA — HOURS POST PARTUM						
		0-10	10-24	24-48	48-72	72-96	96-120	120-144
132	90 mg per os during first 3 days post partum				19	11		
133	90 mg per os during first 3 days post partum					13	12	
134	15 mg. intramuscularly and 30 mg [illegible]				25*	17		
[illegible]	[illegible]							
	post partum							
137	20 mg. per os 20 hours post partum					14 (277)		
73	25 mg per os 2 hours post partum 25 mg per os 24 hours post partum	15* (284)				16 (320)		

*Specimens obtained before administration of vitamin K.

On the basis of our knowledge concerning vitamin K and its effect on the newborn, it seems justifiable to infer from these observations that *vitamin K administered to the mothers in sufficient quantities after delivery is excreted with the mothers' milk in a form still capable of raising the quantity of prothrombin when absorbed from the intestinal tract of the newborn.*

This finding seems to be of essential consequence It has been stated that milk contains little or no vitamin K This may prove to be a relative statement and possibly has to be viewed in direct relation to the diet of the individual or animal whose milk has been subjected to investigation This point is shortly to be touched upon

C *Prenatal Administration of Vitamin K to the Mother.*—In another small series it is shown that *the physiologic hypoprothrombinemia of the newborn can be prevented by administration of vitamin K to the mother before delivery* (Table XLIV)

Of interest is the observation that a large dose of vitamin K to the mother given not later than eleven days before delivery exerts a less pronounced stimulation of the prothrombin production in the newborn as compared to an

TABLE XLIV

THE PROTHROMBIN CONTENT OF NORMAL NEWBORN INFANTS AT VARYING INTERVALS POST PARTUM AFTER ADMINISTRATION OF VITAMIN K TO THE MOTHERS BEFORE DELIVERY

	11 days prepartum							
129	Naphthoquinone, 500 mg. per os for 10 days; continued up to time of delivery	313					332	
[illegible]	[illegible]							
	7 hours prepartum							

*Regular prothrombin time in seconds.
†This specimen was obtained *before* first breast feeding was given.

identical dose given not later than six days before delivery (Cases 127 and 128). This may mean that vitamin K when given in surplus is not stored but gradually is excreted or metabolized in the organism of the mother; further, that this is to a certain extent a time-consuming process. From Table XLIII it may be noted the excretion through the milk is started within a few hours (Case 134). These observations intimate that vitamin K in the organism of the mothers is either a fairly stable substance or that the dosage employed here represents a huge excess as compared to the normal quantity present in the blood, or a combination of both

Where vitamin K medication is continued in sufficient doses up to the time of delivery a stimulation to full production of prothrombin ensues (Case 129). The favorable results in this instance may no doubt partly be attributed to the conveyance of vitamin K to the infant through breast feedings By the same reason it is apparent that satisfactory results can likewise be obtained by administration of vitamin K in larger doses to the mother only a few hours before delivery (Case 131).

Of particular interest is the observation in Case 130. Vitamin K in a single dose of 50 mg. 7 hours before delivery resulted in a normal quantity of prothrombin of the infant 1 hour after delivery. During the following 24 hours *the infant did not receive any breast feedings from the mother. It is to be noted that during this interval the quantity of prothrombin was reduced from 340 to 218 units per cubic centimeter of plasma, indicating that an initial reduction in quantities of prothrombin was not counteracted by a sufficient production of prothrombin to maintain its original level This single observation seems to me to crystallize the results of other observations already presented or to follow, namely, that a constant and sufficient supply of vitamin K is required by*

the organism of the newborn between the first and the sixth day of life in order to uphold an adequate production of prothrombin. In the case under particular consideration we know that any further reduction in the quantities of prothrombin must have been prevented with the extra supply of vitamin K being made available with the onset of the breast feedings.

Investigation of the quantities of prothrombin of the mothers before the onset of vitamin K medication in four patients (Cases 126, 127, 128, and 129) revealed normal values with the two-stage titration technique. With the discontinuation of the medication as stated in Table XLIV, it was found that the quantity of prothrombin had not been increased above normal value in spite of large doses of vitamin K.

From the results listed in Tables XLII, XLIII, and XLIV important inferences can be drawn. With sufficient quantities of vitamin K in one way or the other made available to the newborn, the quantity of prothrombin will remain within, or can be made to reach, normal values. In other words, *under stated conditions the organism of the newborn (the liver) is able to produce quantities of prothrombin equal to that of the normal adult. This excludes the possibility that the physiologic or the excessive hypoprothrombinemia results from a functional inability to produce prothrombin in normal quantities.* Coupled with observations considered above, this strongly indicates that *the physiologic and excessive hypoprothrombinemia results from a quantitative or qualitative deficiency of vitamin K. Excessive quantities of vitamin K in the mother or the infant do not create a hyperprothrombinemia*

Comment.—Experiences with the group of hemorrhages in the newborn indicated that vitamin K therapy corrected both the excessive and the physiologic hypoprothrombinemia. I repeat this again as it goes to show that the difference in the two instances is one of degree only. It more than suggests that the response of the physiologic hypoprothrombinemia to vitamin K medication in the present series, whether the vitamin is given to the mother before delivery or to the mother or infant after delivery, can be used as a criterion to indicate that an excessive hypoprothrombinemia also may be prevented with this type of treatment. Thus is obtained the rational basis for a prevention of hypothrombinemia hemorrhagica neonatorum

The question remains, which of the employed types of vitamin K medication seems the most promising for a successful preventive regimen? A consideration of this essential point must necessarily also include factors, other than those of hematologic nature, known to be of paramount importance in the creation of hemorrhagic manifestations in the newborn. A suggestion as to a convenient preventive regimen may therefore await further consideration after additional data have been presented.

Finally a word concerning the prothrombin production by the liver. The full weight of data concerning vitamin K and prothrombin indicates that in the presence of a K-avitaminosis or a K-hypovitaminosis the liver reacts by a specific impairment in its function of prothrombin production We do not know the level of this critical quantity of vitamin K which is just high enough to exert full

stimulation of the liver to prothrombin production. From the above findings it appears that quantities of vitamin K above this level, however, do not drive the liver to excessive formation of prothrombin.

6. The Mechanism of the Hypoprothrombinemia in the Newborn.—In the part dealing with hemorrhages in jaundiced cases a special section was superfluous for the understanding of the hypoprothrombinemia in those cases. The fat-solubility of vitamin K explains that it could not be satisfactorily absorbed in the absence of bile acids. In the newborn we are not in the position to give such a clear-cut explanation. What is the mechanism of hypoprothrombinemia in the latter cases?

For the sake of clarity let us again state the basis for the further consideration; namely, the peculiar pattern of the initial drop in prothrombin soon after birth, the sustained low level during the first to the sixth day of life, and the subsequent rise in concentrations of prothrombin, a postnatal reaction which by all signs appears to express a physiologic reaction. Further are to be considered the facts that cases with hemorrhage have an excessive hypoprothrombinemia, and that hemorrhages in the majority occur during the period characteristic of physiologic hypoprothrombinemia in normal newborn children.

Considering the doubtless simpler physiologic reaction first, what other physiologic phenomena in the newborn can likely be viewed in relation to changes in the prothrombin? Here one in particular is to be considered; namely, the alleged bacterial inactivity of the intestinal flora in the newborn immediately after delivery. Its significance in this connection results from the observations that a hypoprothrombinemia cannot be produced in the normal adult on a diet that does not contain vitamin K[31] The bacterial activity of the intestinal flora constitutes a sufficient producer of vitamin K.[32]

To prove that the transitory hypoprothrombinemia in the newborn results from an inactive bacterial flora constitutes a problem of no small magnitude, as will readily be understood. An elaborate, detailed study of this problem was not undertaken. In cooperation with Salomonsen a series of observations was recorded which primarily aimed at a rough orientation as to the present question.

A few preliminary remarks are warranted. At the Obstetrical Department it has been customary in the majority of the cases to depend solely upon breast feedings when such is possible. The very first breast feeding has been given from 12 to 20 hours after delivery. Under normal conditions additions to the breast feedings have not been given during the first week of life

Did the initial period of inanition have any relation to the initial drop of prothrombin, as recorded in previous tables? It was decided to investigate two comparable groups of newborns, one group being on the standard regimen, the other group being placed on extra feedings The cases were unselected. The investigation was carried out during the months of August, September, and the first half of October, a fact which may have some bearing on the recorded results

Group 1 consisted of twelve healthy, apparently normal infants on the standard dietary regimen. Group 2 was comprised of thirteen infants who

were all started 2 hours after delivery on extra feedings of one-half diluted cow's milk that had been boiled. During the first day of life 10 c.c. of diluted cow's milk was given six times daily, increasing with 10 c c. per feeding each day for 4 days. During this time breast feedings were also given as for Group 1. After the fourth day breast feedings only were also given to Group 2 children.

Recordings of the prothrombin time (Table XLV) for the cases in Group 1 are identical to the previous findings tabulated in Table XXXV; namely, the development of a transitory prolongation during the first 5 days post partum. This is in contrast to the prothrombin time in the cases of Group 2, in which the prothrombin time in all except one remained within the normal limits.

TABLE XLV

ACCOUNT OF PROTHROMBIN TIME IN NORMAL NEWBORN INFANTS AT VARYING INTERVALS POST PARTUM UNDER TWO DIFFERENT DIETARY REGIMENS

	PROTHROMBIN TIME (SECONDS) HOURS POST PARTUM			
	24-48	48-72	72-96	96-120
Group 1				
Regular breast feeding from 12 to 20 hours	28	30	17	27
post partum	30	33	29	
(12 observations in 12 cases)		35	29	
		35	32	
			40	
Group 2				
In addition to above regimen, extra feedings	17	15	16	17
given from 2 hours post partum	20	15		
(10 observations in 10 cases)	28	15		
		20		
		20		

Daily determinations of the coagulation time of whole blood according to the method of Sabraze were performed in every case of both groups The average of these readings for each day and group is given in Table XLVI.

TABLE XLVI

ACCOUNT OF COAGULATION TIME OF CAPILLARY WHOLE BLOOD IN NORMAL NEWBORN INFANTS AT VARYING INTERVALS POST PARTUM UNDER TWO DIFFERENT DIETARY REGIMENS

	AVERAGE COAGULATION TIME (MINUTES) (CAPILLARY METHOD) DAYS POST PARTUM							
	1	2	3	4	5	6	7	8
Group 1 (12 cases) Average initial loss of weight, 7.3 per cent	3.0	3.6	4.1	3.9	3.6	3.6	3.4	3.2
Group 2 (13 cases) Average initial loss of weight, 3.4 per cent	2.7	3.2	3.0	2.7	2.8	2.7	2.6	2.6

It appears that extra feedings of the infants from within 2 hours after delivery may prevent the development of the prolongation of the prothrombin time during the first week of life. In three cases in which quantitative deter-

mination of prothrombin was performed, a definite hypoprothrombinemia was found to exist, although it was moderate enough to effect a prothrombin time within normal limits.

In three cases sips of sterile water only were given during the first 30 to 34 hours, after which time regular breast feeding was instituted (Table XLVII). In two patients (Cases 154 and 155) it will be noted that the prothrombin time at the end of the period of relative inanition was not any more prolonged than that in the cases of Group 1. In a third patient (Case 156) for some reason the fact was overlooked that the infant received practically nothing from the mother, which was not called to our attention before about 80 hours post partum. The prothrombin time was then found to have increased from 25 to 65 seconds since 28 hours after birth. The coagulation time of whole blood was found to be 7 minutes. *It may be mentioned that this is the first case encountered among all* the cases investigated in which the prothrombin time in the newborn exceeded 50 seconds without any clinical manifestation of hemorrhages With this in mind vitamin K was immediately given. No further sinus puncture was deemed advisable The following day the coagulation time was found within normal limits. The further progress of the infant was fortunately satisfactory in every respect. With the mother the child was dismissed in very good general condition 11 days after birth We admit that the experience here related has checked any desire we may have harbored in extending the present investigations to others on a similar regimen

TABLE XLVII

COAGULATION TIME OF WHOLE BLOOD IN 3 NORMAL NEWBORN INFANTS ON RESTRICTED FEEDINGS POST PARTUM

CASE	DIETARY REGIMEN	COAGULATION TIME (MINUTES) (CAPILLARY METHOD OF SABRAZE) FIGURES IN PARENTHESES INDICATE PROTHROMBIN TIME (SECONDS)							
		HOURS POST PARTUM							
		0-24	24-48	49-72	72-96	96-120	120-144	144-168	168-192
154	Sips of water during first 30 hours	4½	5½ (30)	5	5½ (30)		4½	4	3½
155	Sips of water during first 34 hours	4½	6 (28)	6½	6½	5½ (27)	4½	3½	
156	As above, only little received from mother during next 46 hours		4 (25)	5½	7* (65)	3	2½	3½	3

*Vitamin K therapy instituted immediately after finding of the prolonged prothrombin time

Comment.—In writing up a preliminary report[33] concerning these findings we felt at the time that they suggested a hastening of the bacterial metabolism in the intestines of the newborn who were started on extra feedings immediately after delivery, thus creating a supply of vitamin K sufficient to prevent hypoprothrombinemia. This inference seemed warranted to us at the time It will be readily understood that it silently implies that no vitamin K is contained in the extra feedings of Group 2 This admittedly is an assumption. It was based on a statement that milk contains little, if any, vitamin K. From our experience with regular breast *feedings it has been evident that physiologic hypo-*

prothrombinemia continues at least 3 days after the breast feedings were started, observations indicating that the mothers' milk contains little or no vitamin K. That the latter can be made to excrete vitamin K is now evident from the investigation with administration of the vitamin to the mothers after delivery. By analogy, is it not possible that variations in the vitamin K content of cow's milk may be brought about in a similar manner; that is, being dependent on variations in the vitamin K content of the fodder? It is to be remembered that the investigations were carried out in the months of late summer. For these reasons it seems to suggest that the differences in the prothrombin time noted in the two groups on different dietary regimens may be brought about by small quantities of vitamin K contained in the cow's milk. The boiling of the milk would not have reduced the quantity of vitamin K as, according to Almquist,[33] it is fairly heat stable. In repeating a similar type of investigation an adequate basis for conclusions can be obtained only by simultaneous assaying of the vitamin K content of the liquid diets of the two comparable groups. As this was not performed in the above-presented investigation, the positive information rendered by the observations loses much of its significance. The negative side of the picture is, however, still not influenced by these reservations A moderate period of inanition does not bring about a marked hypoprothrombinemia, although this may result from a more prolonged period of inanition. *Together with the constant finding of a secondary rise in the prothrombin about the end of the first week, this suggests that the activity of the intestinal flora of the newborn is of consequence for the physiologic variation in the concentration of prothrombin during the first week of life*

It seems rather clear, however, that this view in no way explains the excessive degrees of hypoprothrombinemia as characteristic for the cases in which there are hemorrhagic manifestations It appears that one or more additional factors have been introduced, resulting in a parallel displacement of the postnatal prothrombin variations toward lower quantitative values while maintaining the characteristic postnatal pattern

The nature of this factor or factors is not known It may be of some consequence, however, for the further elucidation of the problem that certain logical possibilities be discussed in direct connection with the preceding observations

From a purely theoretical viewpoint at least three possibilities present themselves as reasonably concerned with the creation of an excessive hypoprothrombinemia in the infant

1 An abnormally low concentration of prothrombin or vitamin K in the blood of the infant at the time of birth

2. An excessive consumption of prothrombin during the first hours and days post partum

3. An abnormally low dietary supply of vitamin K during first days of life.

Let us consider these possibilities in the order mentioned. The rationale of the first possibility is indicated by certain observations·

During investigations of the dietary hemorrhagic disease of chicks Almquist and co-workers[33] noted that hens kept on a diet poor in vitamin K produced

chicks which more readily exhibited manifestations of hemorrhage as compared to chicks from hens kept on a diet rich in vitamin K. Almquist's conclusion was that in fowls the vitamin K is transferred from the diet of the mother to its offspring. In passing, it may be recalled that previous findings indicate this to hold true also in the case of human beings. It is, however, the negative side of the question in which we are particularly interested at the moment; namely, that vitamin K, if not transmitted in certain quantities, renders the offspring less protected against hemorrhages.

A direct parallel to this in man may be rare for one reason in particular. The metabolic activity of the intestinal flora in adult human beings guarantees under normal conditions a sufficient endogenous delivery of vitamin K. Fowls appear to a greater extent to lack a sufficient endogenous production due to their relatively short colon. Under pathologic conditions in man this situation may be completely altered and so furnish a fairly parallel picture to that observed by Almquist and his associates. The following case may illustrate this relationship

Case Report.—A woman, 37 years of age, had had a radical operation for carcinoma of the breast during the autumn of 1938. Subsequently she became pregnant. The course of the pregnancy was not unusual until the last month before the expected term when she noted the onset of weakness, loss of weight, and increasing jaundice A physician was not called in until after the onset of labor, at which time she was immediately admitted to the hospital. On examination it was noted that the liver was greatly enlarged and its surface was studded with palpable hard tumors She had a marked degree of jaundice, secondary anemia, and a crop of petechiae and subcutaneous hematomas over the lower extremities. She was emaciated; the presence of ascites was ascertained after delivery. The clinical diagnosis was metastasis to the liver and peritoneum. The delivery was spontaneous and normal. The full-term baby appeared well during the first hours after birth. About 14 hours after delivery, however, the picture was totally changed and all the clinical signs of a cerebral hemorrhage were present. Simultaneously a hemorrhagic infiltration comprising the entire scalp was noted. Several large hematomas over the shoulders and upper part of one arm had also appeared The general condition rapidly became worse in spite of an emergency blood transfusion, the father acting as donor. The child failed rapidly and died the second day after birth; hematologic observations were not performed

I believe this case leaves little doubt as to the close relation between the prothrombin level of the mother and that of the infant. In this case it may be justifiable to state that the hypoprothrombinemia was of congenital origin, although Whipple's contention may still be defended, that its excessive degree probably also was a postnatal phenomenon

It is clear that this case is of little significance with regard to regular cases of hemorrhage in the newborn. The prothrombin of the mothers in the present series does not essentially differ in quantity from that of normal This, however, is not synonymous with a normal concentration of vitamin K, as normal quantities of prothrombin do not express quantities of vitamin K It only indicates that vitamin K is made available to the liver in concentrations equal to or exceeding a certain as yet unknown minimal requirement for normal production of prothrombin. What is above this level constitutes a surplus so far as prothrombin production of the mother is concerned. We do not know

whether it also constitutes a surplus as far as the requirement for vitamin K of the offspring is concerned. Indirect evidence seems to show that the total quantity of vitamin K of the mother is reflected in the postnatal course of the infant's prothrombin, as is observed in the fowl. In this direction is pointed out the seasonal variation in the physiologic hypoprothrombinemia and readily is understood on the basis of varying supply of vitamin K in the mother's diet Granted that such is the case, it is still clear that species with long intestinal tracts do not reveal a K-avitaminosis even following inanition. In other words, variations in the concentration of vitamin K of the mother are likely to occur on a dietary basis. A dietary K-avitaminosis comparable to a C-avitaminosis does not exist.

It seems, therefore, that other factors must be considered active in producing the excessive hypoprothrombinemia in the infant. It is suggestive that the vitamin or the prothrombin may be influenced by intoxication of pregnancy, by medical regimens like the Stroganoff treatment, by repeated courses of quinine, or through hormonal effects.

With regard to the second possibility listed, we do not know any more about excessive prothrombin consumption through deposition of fibrin in the present group as compared to that of obstructive jaundice.

As to the lack of a sufficient, constant supply of vitamin K to the infant during the first days of life, it is to be kept in mind that all the factors exerting an unfavorable influence on the vitamin K of the mother may also be reflected in a lower vitamin K content of the mother's milk. *The infant may thus be doubly exposed to the influence of these factors.* It seems probable that an approach to a study of these points may be afforded by an assay of the vitamin K concentrations of the mother's milk under various conditions

In summarizing, it may be stated that *the physiologic hypoprothrombinemia in the newborn is reasonably explained on the basis of an inactivity of the bacterial flora of the intestines during the first days of life This, however, does not explain the excessive hypoprothrombinemia in cases of hemorrhage. Additional factors of still unknown nature must be assumed to be superimposed upon the physiologic reaction leading to the moderate hypoprothrombinemia present in all normal newborn infants. It is still an open question whether the excessive hypoprothrombinemia is primarily of maternal origin or develops solely as an excessive postnatal reaction in the newborn or a combination of both.*

In spite of admitted lack of complete observations I have ventured to carry the discussion thus far for one main reason In considering a regimen for the prevention of hemorrhage in the newborn through vitamin K medication, the above-presented questions, even if not answered at present, must be duly considered Essential factors of nonhematologic nature must further be accounted for

7. The Mechanism of Hemorrhage in the Newborn.—In the three preceding main chapters dealing with hemorrhage in hemophilia, in thrombocytopenic purpura, and in diseases of the bile ducts and the liver I have intimated that an

chicks which more readily exhibited manifestations of hemorrhage as compared to chicks from hens kept on a diet rich in vitamin K. Almquist's conclusion was that in fowls the vitamin K is transferred from the diet of the mother to its offspring. In passing, it may be recalled that previous findings indicate this to hold true also in the case of human beings It is, however, the negative side of the question in which we are particularly interested at the moment; namely, that vitamin K, if not transmitted in certain quantities, renders the offspring less protected against hemorrhages.

A direct parallel to this in man may be rare for one reason in particular. The metabolic activity of the intestinal flora in adult human beings guarantees under normal conditions a sufficient endogenous delivery of vitamin K. Fowls appear to a greater extent to lack a sufficient endogenous production due to their relatively short colon. Under pathologic conditions in man this situation may be completely altered and so furnish a fairly parallel picture to that observed by Almquist and his associates. The following case may illustrate this relationship.

Case Report.—A woman, 37 years of age, had had a radical operation for carcinoma of the breast during the autumn of 1938. Subsequently she became pregnant. The course of the pregnancy was not unusual until the last month before the expected term when she noted the onset of weakness, loss of weight, and increasing jaundice. A physician was not called in until after the onset of labor, at which time she was immediately admitted to the hospital. On examination it was noted that the liver was greatly enlarged and its surface was studded with palpable hard tumors. She had a marked degree of jaundice, secondary anemia, and a crop of petechiae and subcutaneous hematomas over the lower extremities She was emaciated, the presence of ascites was ascertained after delivery. The clinical diagnosis was metastasis to the liver and peritoneum. The delivery was spontaneous and normal. The full-term baby appeared well during the first hours after birth. About 14 hours after delivery, however, the picture was totally changed and all the clinical signs of a cerebral hemorrhage were present. Simultaneously a hemorrhagic infiltration comprising the entire scalp was noted. Several large hematomas over the shoulders and upper part of one arm had also appeared. The general condition rapidly became worse in spite of an emergency blood transfusion, the father acting as donor. The child failed rapidly and died the second day after birth; hematologic observations were not performed.

I believe this case leaves little doubt as to the close relation between the prothrombin level of the mother and that of the infant. In this case it may be justifiable to state that the hypoprothrombinemia was of congenital origin, although Whipple's contention may still be defended, that its excessive degree probably also was a postnatal phenomenon.

It is clear that this case is of little significance with regard to regular cases of hemorrhage in the newborn The prothrombin of the mothers in the present series does not essentially differ in quantity from that of normal. This, however, is not synonymous with a normal concentration of vitamin K, as normal quantities of prothrombin do not express quantities of vitamin K. It only indicates that vitamin K is made available to the liver in concentrations equal to or exceeding a certain as yet unknown minimal requirement for normal production of prothrombin. What is above this level constitutes a surplus so far as prothrombin production of the mother is concerned. We do not know

tions may not exhibit these phenomena. In both instances the traumatic capillary derangement due to pressure from the blades of the forceps may be fairly comparable. The essential difference between the two cases is the physiologic hypoprothrombinemia in the latter case as compared to the excessive hypoprothrombinemia in the former.

The manifestation described is most instructive. Capillary functional deficiency does not in itself lead to hemorrhagic manifestations. For the creation of hemorrhages another factor is required; namely, the presence of hypocoagulability of the blood. If it is taken into consideration that the majority of hemorrhages in the newborn occur during the period of physiologic hypoprothrombinemia in the normal infant, furthermore, the frequently discussed free interval between birth and the onset of hemorrhages, it will readily be appreciated that *the hematologic factor must be considered of paramount importance* for an understanding of the processes leading to bleeding in the newborn

It is concluded that, *irrespective of the traumatic lesion of the capillaries of whatever origin and localization, the subsequent hemorrhagic manifestations appear with the development of an excessive hypoprothrombinemia. On this basis is founded the reasonable expectation that hemorrhages in the newborn are preventable through Vitamin K medication.*

In order to simplify matters the discussion has been brought thus far without intimating that hemorrhages in the newborn constitute a well-defined, homogenous group as depicted, with essential reservations A complete review of these reservations, particularly concerning melena and cerebral hemorrhages, will be omitted. For clarification of Part 8 of this chapter, however, it is necessary to consider certain points relative to cerebral hemorrhage

It seems fairly clear that a certain number of cerebral hemorrhages are understood to occur on the basis of the above-suggested mechanism, that is, upon the development of excessive hypoprothrombinemia in the presence of traumatic disturbances of small or large capillary areas. These cases are characterized by the much discussed free interval between the delivery and the onset of the clinical manifestations of cerebral pathologic changes. These cases naturally take their place among the other hemorrhagic manifestations of hypothrombinemia hemorrhagica neonatorum. There is every reason to expect that their response to a preventive regimen also based on vitamin K will prove to be identical to the remainder of the group

Cerebral hemorrhages, however, occur in a certain percentage of cases with no distinct free interval; that is, the cerebral hematoma is formed during or immediately after delivery In fifty-six out of eighty-four cerebral hemorrhages Salomonsen noted the onset of the first clinical symptoms during the first day after delivery, and in only one of them did they occur in connection with a prolonged coagulation time of whole blood If these figures can be considered representative for the general relation between the immediate and the delayed types of cerebral hemorrhage, we may at this point clearly vision serious limitations to the preventive effect of vitamin K in hemorrhages of the newborn.

existing hypocoagulability of the blood represents *only* one, although an important, phase of the problem. This will presently be considered in detail

As *previously stated,* extreme hypocoagulability of the blood may exist for a considerable time before actual hemorrhage occurs Extravascular *accumulation of blood is the result* of hematologic and vascular factors. As discussed earlier, evidence points to the *intimacy* between the *functional activity of the* vessels and the blood. In certain instances vascular changes alone may possibly lay *the ground for hemorrhage in spite of normal blood coagulability. In the* majority of the cases under discussion both factors appear to operate side by side. The agent *precipitating* the *hemorrhages, however, is nonvascular and* nonhematologic. This third factor is trauma. When discussing the influence of this factor in connection with the *present cases, it is to be stressed that trauma* of importance to the vascular tree is of quite a different order than that leading *to, for example, fracture of bones. A few examples may clarify the point:*

The spontaneous hemorrhages into various joints in the hemophiliac, the *subcutaneous* hemorrhages under garters and wrist watches in thrombocytopenic purpura, hematuria in jaundiced cases, and subcutaneous hemorrhages following excessive sneezing and coughing in any of the groups have one thing in common. The hemorrhagic manifestations occur upon repeated or single, voluntary or involuntary movements of the body which in *the normal person* would not lead to hemorrhages and could not be considered to represent trauma in the regular sense of the word. In the same group of phenomena are found the frequently recorded crops of pericardial and pleural petechiae caused by the rhythmic contraction and expansion of the lungs and the heart.

In the newborn can be seen the whole register of traumatic influences, from the more severe ones caused by forceps to the minute trauma that produces pericardial and pleural petechiae, bleeding from dermal fissures where the skin has been exposed to excessive *bending or stretching, the subcutaneous hemorrhages* at the tips of the fingers following scratching, or at the tips of the toes due to *kicking against* the bedclothes.

In each instance the traumatic factor appears primarily to create a lesion or *functional derangement of* the capillaries which secondarily leads to an extravascular accumulation of blood if the hematologic factor is present. We are again here confronted with the old problem whether the vascular or the hematologic factor is of primary importance. I believe this *cannot* be *answered* at present. Under present conditions, however, *observations indicate that hemorrhages occur on a hematologic basis when a definite vascular abnormality is previously brought about by external trauma* The sequence of events is well demonstrated by the following clinical phenomenon which is not a rare occurrence in any obstetrical department

An infant, delivered by forceps, appears by every external sign to be doing perfectly well. About the second to the *fourth day after birth* hemorrhagic infiltrations of both cheeks may be noted, the infiltrations clearly tracing the placement of the forceps *Another* child delivered under comparable condi-

In case the vitamin cannot be given to the mother closer to the time of delivery, the findings indicate the advisability of raising the dosage of the vitamin accordingly. As is apparent from Table XLIV a satisfactory effect can be obtained by 50 mg. naphthoquinone administered to the mother a few hours before delivery.

In cases in which intoxication with nausea or vomiting or other conditions makes oral administration less desirable, intramuscular injections in identical doses may conveniently be resorted to.

In communities that have well-organized clinics for prenatal examination and education, the execution of a similar, simple regimen may prove valuable

If for some reason or other vitamin K has not been given to the mother, the next best thing is to administer it to the infant. In order to obtain the important prolonged effect and simultaneously shorten the unavoidable lag period, it may be administered in doses of 5 to 10 mg. by intramuscular injections immediately after delivery and by mouth within the first few hours

On the basis of previously discussed results it seems of importance not to prolong unduly the immediate postnatal period of inanition but institute early extra feedings in small quantities Exact information as to the importance of the latter scheme must necessarily be based on a comparable investigation of the vitamin K content of the milk of mothers and cows

Conclusions

1. During the first 10 hours after delivery the concentration of prothrombin in the blood of the normal infants exhibits subnormal values. From the second half of first day to the sixth day of life there is an additional reduction in quantities of prothrombin, reaching its maximum between 48 and 96 hours post partum. This is followed by a secondary rise from the sixth day post partum. The transitory hypoprothrombinemia constitutes a physiologic, postnatal reaction, closely related to the postnatal inactivity of the intestinal flora

2 The physiologic hypoprothrombinemia is more pronounced during the months of late winter, spring, and early summer as compared to the rest of the year. This difference may be brought about by inherent variations in the vitamin K content of the diet

3 Hemorrhages in the newborn are found to be associated with a hypoprothrombinemia exceeding that considered to be physiologic for this particular stage of life This condition has been termed *hypothrombinemia hemorrhagica neonatorum.*

4 Hemorrhages in the newborn are produced through the combination of an excessive hypoprothrombinemia and a vascular deficiency brought about through traumatic influences This satisfactorily explains that the majority of hemorrhages in the newborn occur between the first and the sixth days of life.

5 The excessive hypoprothrombinemia is not satisfactorily explained solely on the basis of a postnatal inactivity of the intestinal flora. The presence of

By closer analysis of the problem it is not excluded that vitamin K in these cases may also prove of some consequence, and for the following reason:

There is reason to believe that these early cerebral hemorrhages result from trauma exerted upon the infant's head during delivery, and severe enough not only to inflict capillary derangement but direct rupture of the vessels, with immediate formation of a hematoma. The magnitude of the hematoma may vary; it may be extensive enough to cause immediate death, or it may only gradually extend through slow oozing of blood into the originally small hematoma. As will be recalled, the concentration of prothrombin in the normal infant must be considered subnormal. We do not know whether certain infants may exhibit a marked hypoprothrombinemia at time of birth. In either instance it is clear that the hemostatic mechanism under these circumstances must be deficient in essential respects and that this deficiency can be corrected by raising the concentration of prothrombin to the normal value. In some instances the vascular discontinuity may be so extensive as to permit free bleeding in spite of a normal hemostatic mechanism of the blood. These cases must be written off as impossible to influence by any preventive medical regimen. Where trauma is less extensive and the danger mainly consists of the slow oozing of blood, a more efficient hemostasis may be expected to limit the extent of the hematoma. *Among certain of these borderline cases vitamin K regimen may prove to be a great success; in other cases, to be a disappointment as it may prevent the fatal issue without influencing the morbidity.*

8. Suggestion as to a Vitamin K Regimen for Prevention of Hypothrombinemia Hemorrhagica Neonatorum.—Through the preceding remarks I have stated what with reason can be expected from a medical preventive regimen in the present condition and what is beyond its reach Vitamin K is never going to supplant the skill and judgment of the obstetrician. It may, however, protect the immediate results of his efforts on an important point.

From the previous discussion it seems clear that any preventive regimen hoping to protect the infant from hemorrhages during delivery as well as during the immediate postnatal course must be based on the creation of a maximal concentration of prothrombin in the infant at the time of delivery. This excludes any other possibility than administration of vitamin K to the mother before delivery.

Such a regimen can readily be carried out through cooperation with the mother. Believing, as I have stated, that the synthetic preparations eventually will supplant the purified extracts of vitamin K, a reasonable regimen would be based on the administration of 2-methyl-1,4-naphthoquinone, one tablet of 5 mg. twice daily during the last 2 weeks of pregnancy. For obvious reasons this medication is to be carried out regardless of whether or not the mother intends to or will be able to give breast feedings If breast feedings are not given it seems indicated also to administer vitamin K to the infant immediately after delivery, while this is superfluous if breast feedings are given by a mother who had received sufficient quantities of vitamin K.

day post partum rather profuse hemorrhage from the infant's umbilicus was noted which necessitated blood transfusion (15 c c. citrated blood). The general condition was satisfactory; bleeding was arrested rapidly and the further course was normal.

CASE 141.—A quartipara, aged 39 years, had had much vomiting and nausea during pregnancy. During the last 2 months she had had access to no other vegetables than potatoes. She drank one and one-half quarts of milk daily. During one month's stay in hospital she was given several courses of quinine treatment because delivery was overdue. Delivery on Aug 3, 1939, was normal. About 45 hours post partum severe hemorrhage from the infant's umbilicus occurred which could not be arrested by regular local treatment. (Previously the mother had requested special attention be directed to this possibility as navel hemorrhages had occurred in her three previous children) A subcutaneous hematoma was noted over the temporal region and over the costal margin. After 4 hours of oozing the baby looked rather pale. Vitamin K treatment was begun; 50,000 units (Dam) of purified extract were given intramuscularly and 100,000 units per os during the next few hours The navel was dressed after 2 hours and the bleeding had ceased then. There was no further evidence of hemorrhage The child gradually recovered from the secondary anemia and the general condition otherwise was very satisfactory.

CASE 142.—A primipara, aged 21 years, had had a normal pregnancy and delivery on March 4, 1939 The child was full term. During the second day of the infant's life convulsions of the facial muscles, arms, and legs occurred. The muscles were spastic. The following day convulsions continued and difficult breathing and bulging fontanel developed. A transfusion of 20 c c citrated blood was given, with resultant slow improvement. Upon dismissal there were definite signs of cerebral pathologic changes

CASE 143.—A primipara, aged 26 years, the day before admission to the hospital May 9, 1939, had noted slight edema of the ankles She had moderate hypertension and albuminuria and was put on salt-free diet Quinine treatment was also instituted On May 14 she had two attacks of eclampsia; the Stroganoff treatment was instituted. Next morning the child was delivered by forceps, it was a full term infant. After 48 hours large hemorrhagic infiltration was noted on both cheeks at site of forceps blades There was also a small hematoma of the chin and neck and bleeding scratch marks on the face. Soon after, bleeding from navel began. A transfusion of 20 c.c. of citrated blood was given (May 17). The oozing from the navel and scratch marks soon ceased Next day the child appeared rather sleepy and inactive, otherwise the condition was satisfactory. Bleeding from navel, dermal fissures, and scratch marks recurred on May 19 A second transfusion (20 c c. citrated blood) checked further bleeding tendency, and the child was dismissed in good condition

CASE 144—A secundipara, aged 30 years, had had a normal pregnancy and delivery May 2, 1939, at full term. About 48 hours post-partum crops of petechiae appeared on the face of the infant There were bleeding scratch marks on the face and from dermal fissures in the inguinal region The bleeding was checked by transfusion (20 c c citrated blood). The further course of the child was satisfactory.

CASE 145—A primipara, aged 26 years, had had a normal pregnancy and delivery May 5, 1939. Forceps were used because of the slow progress and rapid fetal heart tones The baby was full term. On the fifth day post partum the baby exhibited hemorrhagic infiltration of marks of forceps blades There was a hematoma of the left shoulder and the upper arm, also cephalic hematoma. Bleeding dermal fissures were present on the dorsum of both feet. A transfusion of 20 c c. of citrated blood was given, and bleeding from dermal fissures was arrested soon afterward with blanching and disappearance of subcutaneous infiltrations during the following days. Further course of the child was satisfactory

CASE 146—A primipara, aged 37 years, was admitted April 19, 1939. Her blood pressure was 190 systolic and 120 diastolic. Albuminuria was not present. While in hospital the

additional superimposed factors is postulated. Whether these are primarily of maternal origin or developing as excessive postnatal reaction, or a combination of both, remains unanswered.

6. Blood transfusions in hypothrombinemia hemorrhagica neonatorum exert their antihemorrhagic activity through the substitution of prothrombin.

7. Vitamin K represents in these cases a curative principle. It corrects within a few hours the existing hematologic deficiency through the restitution of a normal production of prothrombin by the liver.

8. The development of a transitory hypoprothrombinemia in the newborn can be prevented through the administration of vitamin K to the infant or the nursing mother right after delivery, or to the mother before delivery.

9. Vitamin K administered to mothers in larger doses is excreted with the milk and exerts its normal action in the infant when normally absorbed from the intestines

10. A medical regimen for prevention of hypothrombinemia hemorrhagica neonatorum must aim at the creation of a maximal concentration of prothrombin in the blood of the infant before onset of the delivery. This can be accomplished *through the administration of vitamin K to the mother for several days before* the expected term The preventive regimen must further assure a constant supply of vitamin K to the newborn during the first week of life, either through the breast feedings of the mothers who have been on vitamin K medication, or through direct administration of vitamin K to the infant immediately after birth

11. It is to be expected that vitamin K will prove ineffective in cases in which the traumatic effect has resulted in hemorrhages not essentially influenced by normal hemostatic ability of the blood

Report of Cases

Case 138.—A primipara, aged 32 years, had been vomiting during her entire pregnancy The vomiting ceased 3 months before delivery. The infant was normally delivered at full term, Aug. 1, 1939. Soon after delivery a small blue spot was noted over the left elbow of the infant Twelve hours later numerous petechiae on both eyelids, lower part of abdomen, upper part of arms and thighs were observed A subcutaneous hematoma appeared over the temporal region, in the axilla, over the lower part of the back and buttocks. The stools contained fresh blood. The child's general condition was satisfactory and the blood picture and bleeding time were normal Vitamin K, a purified extract, 50,000 units intramuscularly and 40,000 units per os during next few hours were given Subsequently there were no new hemorrhagic manifestations, while the ones present blanched rapidly. The further course was normal

Case 139.—A secundipara, aged 40 years, had had a normal pregnancy and delivery had been normal March 9, 1939, with the infant born at full term. The first bloody stool was noted about 50 hours post partum and was followed by several others The infant was given 15 c.c. of citrated blood into the longitudinal sinus. During the next 10 hours there were several more bloody stools, the baby appeared restless and the face was cyanosed After 24 hours the melena had stopped. There was fairly rapid improvement in the general condition

Case 140.—A primipara, aged 38 years, had suffered from hypertension and albuminuria during the last 5 weeks of pregnancy Labor was attempted to be induced by six courses of quinine treatment. Delivery occurred May 26, 1939 Forceps were used for delivery because of slow progress of labor. The child was born at full term. During the second

canal which persisted in spite of a plug of cotton in the ear. There were no definite signs of bulging fontanel or convulsions. Vitamin K therapy consisting of 2-methyl 1,4 naphthoquinone, 5 mg. intramuscularly and 5 mg. per os was given Oozing from the ear was arrested. The next day the general condition was much improved and the baby was lively and awake

Twin 2 weighed 2,400 Gm and was more lively than the elder twin. The child throve well during the first two days when constant oozing of blood from navel began which was not arrested by regular local applications. Vitamin K therapy was identical to that for Twin 1. The navel was dressed 7 hours later when oozing of blood was arrested. The further course was satisfactory.

CASE 151.—A secundipara, aged 25 years, had a normal pregnancy and a normal delivery of a full term mongoloid type Oct. 7, 1939. The baby throve until about 72 hours post partum. During the next 24 hours there was slow oozing of blood from the navel, which was not arrested by the application of hemostatic cotton. Vitamin K therapy consisted of 2 methyl-1,4-naphthoquinone, 10 mg intramuscularly and 10 mg. per os The navel bleeding was soon arrested and further hemorrhages did not occur.

CASE 152—A primipara, aged 35 years, had a normal pregnancy with normal delivery of a full-term baby Oct. 2, 1939. During the first one and one half days the baby did well. About 36 hours post partum it was noted that the baby did not take breast feedings and looked stuporous. A few hours afterward there was the onset of convulsions of the musculature of the arms and legs associated with marked cyanosis. The infant was very pale between attacks and cried faintly. The fontanel was slightly tense. Vitamin K therapy consisted of 2 methyl 1,4 naphthoquinone, 5 mg. intramuscularly and 10 mg per os (40 hours post partum) Two hours after vitamin administration the infant had another attack with convulsions and cyanosis of 15 minutes' duration. During the attack almost all reactions were extinguished and the child appeared moribund. After administration of cardiazol and lobelin there was a gradual return of color. Afterward ptosis of the right eye was noted Similar attacks followed during the night and death occurred 49 hours post partum. Permission for post mortem examination was refused.

CASE 153—A primipara, aged 28 years, had been well up to 2 weeks before admission to the hospital when she had onset of headache, edema of the ankles, albuminuria, and hypertension (140 systolic, 105 diastolic) She was admitted to hospital Nov. 17, 1939. She had had two eclamptic attacks at home, and two shorter ones after admission. Albuminuria was 24 per cent and the Stroganoff treatment was instituted. The albuminuria diminished rapidly on a dietary regimen, however, simultaneously there was a gradual increase in blood pressure. When restlessness, nausea, and vomiting began again labor was induced by introduction of the rubber bag She was delivered Dec 12, 1939, of a premature baby of 36 weeks, who weighed 2,130 Gm During the first 5 hours post partum the baby was very cyanotic but the normal color returned spontaneously. After 14 hours slight bleeding from nose and emesis of dark blood were noted. Previously the infant had passed three to four black and bloody stools, one containing bright fresh blood The general condition was not good and moderate pallor was noted Vitamin K therapy consisted of 2-methyl-1,4 naphthoquinone, 10 mg intramuscularly, 5 mg. per os immediately, and 5 mg. twice during the next 24 hours The first normal bowel movement occurred about 34 hours post partum. The further course was satisfactory and the infant took feeding very nicely after onset of improvement. Sinus puncture was not performed because of the poor condition

References

1. Minot, Francis. On Hemorrhage From the Umbilicus in New-Born Infants, With an Analysis of Forty-Six Cases, Am. J. M. Sc 24: 310-320, 1852.
2 Minot, Francis. Spontaneous Haemorrhage in a New-Born Child, Boston M. & S. J. 70: 403-404, 1864.

blood pressure remained high with occasional trace of albumin The woman had her first eclamptic attack May 4, 1939, and Stroganoff treatment was instituted. The woman was delivered on the same day. High forceps were used and the child was born at full term. During the first few days the general condition of baby was satisfactory in every respect. About 96 hours post partum the baby grew increasingly pale and cried faintly; in a few hours there were convulsions of the face and arms, and increasing hemorrhagic infiltration of marks after use of forceps. The fontanel was bulging and respiration slow. Transfusions of 20 c.c. citrated blood were given, but the child died 110 hours post partum. Permission for necropsy was refused.

CASE 147.—A secundipara, aged 27 years, whose pregnancy was normal except for transverse position, was delivered on July 23, 1939, by version and extraction with forceps to aftercoming head. The baby was full term and did well for the first 24 hours when symptoms of cerebral hemorrhage developed; there were repeated episodes of convulsions with deep cyanosis, shallow breathing, and almost complete extinction of the corneal reflex. The fontanel was bulging. Convulsions were brought on by moving or turning the baby, and the condition was very alarming. The child was given vitamin K extract in purified form, 50,000 units (Dam) intramuscularly and 50,000 units by mouth during next few hours. During the following 2 days the condition was still critical with convulsions, after which improvement was gradual and slow. At dismissal there were definite signs of cerebral pathologic changes. Three and one half months later the child was re-examined. The mother stated the baby cried frequently; otherwise she was unable to furnish information of abnormal reactions. Physical examination was essentially negative.

CASE 148.—A primipara, aged 20 years, had a normal pregnancy and was delivered of a full-term baby Aug. 23, 1939. Forceps were used because of slow progress of labor. Up to 48 hours post partum the baby did very well, when moderate pallor and restlessness with faint crying developed There were no convulsions. The fontanel was slightly tense. About 50 hours post partum bleeding occurred from the right ear canal. This stopped after

baby's condition. Previously it had been sleepy, dozing without showing efforts to take feedings. During the following days the infant was normally active and awake, took breast and extra milk feedings without difficulty. At dismissal there were no physical signs of abnormality.

CASE 149.—A primipara, aged 33 years, had been constantly nauseated with frequent vomiting during pregnancy. She was unable to take any other vegetables than carrots. She drank 1 liter of milk daily. Otherwise her condition had been normal. She had a normal delivery of a full-term baby on Sept. 9, 1939. Up to 66 hours post partum the baby did well. At this time onset of oozing from the navel occurred Bleeding dermal fissures over dorsum of both feet and both wrists were also noted. Therapy: 2-methyl-1,4 naphthoquinone, 5 mg. intramuscularly and 5 mg. per os right away. Subsequent course was satisfactory with no new hemorrhages. The navel was dressed after 3 hours when hemorrhage was noted to be arrested.

CASE 150.—A primipara, aged 26 years, during the first 3 months of pregnancy had much nausea and vomiting, later she did very well She ate plenty of vegetables, particularly during the last two weeks of pregnancy because of edema of the ankles and slight albuminuria She was delivered of full term twins Oct. 4, 1939, with the use of forceps because of slow progress of labor.

Twin 1 weighed 3,400 Gm. and was markedly jaundiced and stuporous during the first days. About 68 hours post partum there was moderate oozing of blood from the right ear

28. Whipple, G. H.: Hemorrhagic Disease. Antithrombin and Prothrombin Factors, Ibid. 12: 637 659, 1913.
29. Happ, W. M.: Appearance of Isoagglutinins in Infants and Children, J. Exper. Med. 31: 313 334, 1920.
30. Jones, B. B.: Iso-Agglutinins in the Blood of the New Born, Am. J. Dis. Child. 22: 586-597, 1921.
31. Dam, H.: Vitamin K, Ztschr. f. Vitaminforsch. 8: 248 257, 1939.
32. Almquist, H J., Pentler, C. F., and Mecchi, E.: Synthesis of the Antihemorrhagic Vitamin by Bacteria, Proc. Soc. Exper. Biol. & Med 38. 336-338, 1938.
33 Salomonsen, L., and Nygaard, K. K.: The Prothrombin Content in Relation to Early and Late Feedings of the Newborn, a Preliminary Report, Acta paediat. 27; 209-218, 1939
34. Almquist, H. J.: Purification of the Antihemorrhagic Vitamin, J. Biol. Chem. 114 241-245, 1936
35. Almquist, H. J., Mecchi, E., and Klose, A. A.· Estimation of the Antihaemorrhagic Vitamin, Biochem. J. 32: 1897-1903, 1938.

3. Townsend, C. W.: Haemorrhages in the New-Born, Ibid. 125: 218-220, 1891.
4. Schloss, O. M., and Commiskey, L. J. J.: Spontaneous Hemorrhage in the Newborn; With the Report of Nine Cases, Am. J. Dis. Child. 1: 276 298, 1911.
5. Lovegren, Elis. Erfahrungen und Studien über Melaena neonatorum, Jahrb. f. Kinderh. 78: 249 277, 1913.
6. Lövegren, Elis: Weitere Blutbefunde bei Melaena neonatorum, Ibid. 79: 708-715, 1914.
7. Salomonsen, Leif: Morbus hemorrhagicus neonatorum (hypoprothrombinemia neonatorum), Acta paediat. (suppl. 1) 27: 1-120, 1939.
8. Brinkhous, K. M., Smith, H. P., and Warner, E. D.: Plasma Prothrombin Level in Normal Infancy and in Hemorrhagic Disease of the Newborn, Am. J. M. Sc. 193: 475-480, 1937.
9. Warner, E. D., Brinkhous, K. M., and Smith, H. P.: Bleeding Tendency of Obstructive Jaundice: Prothrombin Deficiency and Dietary Factors, Proc. Soc. Exper. Biol. & Med. 37: 628-630, 1938.
10. Butt, H. R., Snell, A. M., and Osterberg, A. E.: The Use of Vitamin K and Bile in the Treatment of the Hemorrhagic Diathesis in Cases of Jaundice, Proc. Staff Meet., Mayo Clin. 13: 74-77, 1938.
11. Dam, H, and Glavind, J.. Vitamin K i den menneskelige patologi, Ugesk. f. laeger 100: 248-250, 1938.
12. Waddell, W. W., Jr., Guerry, DuPont, III, Bray, W. E., and Kelley, O. R.: Possible Effects of Vitamin K on Prothrombin and Clotting Time in Newly-Born Infants, Proc. Soc. Exper. Biol. & Med. 40: 432-434, 1939.
13. Waddell, W. W., and Guerry, DuPont: Effect of Vitamin K on the Clotting Time of the Prothrombin and the Blood; With Special Reference to Unnatural Bleeding of the Newly Born, J. A. M. A. 112: 2259-2263, 1939
14. Hellman, L. M, and Shettles, L. B.: Factors Influencing Plasma Prothrombin in Newborn Infant, Prematurity and Vitamin K, Bull Johns Hopkins Hosp. 65: 138-141, 1939.
15. Dam, H., Tage-Hansen, E, and Plum, P.: K-avitaminose hos spede børn som aarsag til hemorhagisk diathese, Ugesk. f. laeger. 101: 896-904, 1939
16. Nygaard, K. K.: Prophylactic and Curative Effect of Vitamin K in Hemorrhagic Disease of the Newborn (Hypothrombinemia Hemorrhagica Neonatorum); Preliminary Report, Acta obst. et gynec Scandinav. 19: 361-370, 1939.
17. Owen, C. A., Hoffman, G. R., Ziffren, S. E., and Smith, H. P.. Blood Coagulation During Infancy, Proc. Soc. Exper. Biol. & Med. 41: 181-185, 1939
18. Rodda, F. C.: Studies With a New Method for Determining the Coagulation Time of the Blood in the New-Born, Am J. Dis. Child. 19: 269 276, 1920.
19. Maurizio, Eugenio. Über Pseudohamophilie bei Neugeborenen, Zentralbl. f. Gynäk. 54: 479-484, 1930.
... and Wyatt, J. Substances Involved in Coagulation of
... hrombin in Blood of Babies
... 7-648, 1939.
22. Quick, A. J., and Grossman, A. M.: Prothrombin Concentration in Newborn, Ibid. 41. 227-228, 1939.
... by Lespinasse, V. D., and
... t by Direct Transfusion of
Blood, Surg., Gynec. & Obst. 12: 40-45, 1911
26. Beveridge, Ruby S: Haemorrhagic Disease of the Newborn, Arch. Dis. Child. 3: 39-48, 1928
27. Whipple, G. H: Hemorrhagic Disease—Septicemia, Melena Neonatorum and Hepatic Cirrhosis, Arch. Int. Med. 9: 365 399, 1912.

INDEX

F

G

H

